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Biosorption of copper ions using rhizoplane bacterial isolates isolated from *Eicchornia crassipes* ((Mart.) solms) with kinetic studies

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ABSTRACT

Biosorption of Cu ions from the aqueous solution was studied with *Pseudomonas putida* and *Bacillus cereus*, isolated from rhizoplane region of water hyacinth (*Eichhornia crassipes*) using pure culture technique. The effect of contact time (0, 4, 8, 12, 16, 20, and 24 h), pH (3, 5, 7, and 9), temperature (25, 37, and 45˚C), and initial concentrations of Cu ions (50–1500 mg l^{-1}) on biosorption was investigated in batch systems. Better amount of biosorption of Cu ions (500 mg l^{-1}) from the tested biosorbents was found to be 84.8% by *P. putida* and 83.8% by *B. cereus* at pH 7. The biosorbed Cu ions by *P. putida* and *B. cereus* were 420 and 415 mg l^{-1}, respectively, at 500 mg l^{-1} of Cu ion concentration. The results of pseudo-second-order kinetics were considered as the most appropriate model which predicted the biosorption capacity of both the bacterial isolates with comparison to the experimental results. Langmuir and Freundlich isotherms were used to evaluate the experimental data and their constants were derived. From the data analysis, Freundlich isotherm was fitted well followed by Langmuir isotherm model.

Keywords: Copper ions; Rhizoplane microbes; *Eichhornia crassipes*; Langmuir and Freundlich isotherm models; Pseudo-first and second-order kinetics

1. Introduction

The pollution of the environment with toxic heavy metals is spreading throughout the world along with industrial development. Industrial activity permits the natural flow of materials and introduces toxic chemicals into the environment [1]. Cu is widely used in the industry of electrical equipment and wires, pipes, alloys, antifouling paints, algaecides, and wood preservatives. It is considered the second toxic metal to aquatic organisms after Hg [2]. Toxic chemicals like heavy metals include copper, cadmium, lead, nickel, etc. that contaminate the soils, ground water, sediments, and surface waters and they are present in soluble form. Most of them are extremely toxic to biological systems and ecological activity is disrupted. Traces of these heavy metals are necessary as co-factors of enzymatic reactions, but high levels of them may cause extreme toxicity to living organisms due to inhibition of metabolic reactions [3].

Copper ions released due to the discharge of effluent into the environment by a large number of processes such as electroplating, leather tanning, wood preservative, pulp processing, steel manufacturing,

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etc. and the concentration levels of these heavy metals varies widely in the environment. Conventional physicochemical methods being economically expensive and contain disadvantages like incomplete metal removal, higher reagent, energy requirements, and generation of toxic sludge's and other waste products that require disposal. As a result, many new technologies are required to mitigate Cu ion concentration to environmentally acceptable levels [4].

Biosorption is proven to be quite effective for the removal of Cu ions from contaminated solution because this method requires low cost and is environment friendly [5]. In previous studies, there is a lot of bacterial isolates like *Pseudomonas* sp., *Bacillus* sp., *Aeromonas* sp. [6], *Staphylococcus* sp., and some α-, β-, and *γ-proteobacteria*, *Spingobacteria* [7,8] were used for the biosorption of Cu ions. Some copper-resistant bacteria show abilities of biosorption, bioreduction, bioaccumulation, and bioleaching of copper [9] and can be used in combination with plants to phytoremediate copper from polluted sites [10].

Current investigation was designed to determine the biosorption capacity of Cu ions from aqueous solution using rhizoplane bacterial isolates (*P. putida* and *Bacillus cereus*) of water hyacinth. The effect of pH, temperature, and initial concentration of metal ions on biosorption of Cu ions was evaluated. The resulting data were analyzed with Langmuir and Freundlich isotherms and also with pseudo-first and second-order kinetic models.

2. Materials and methods

2.1. Isolation and identification of rhizoplane bacterial isolates

Water hyacinth was collected from local pond (Pottalputhur, Tamil Nadu, India, 8˚47´58´´N, 77˚18´55´´E) and brought to the laboratory. Plants with pest free, fresh, without off shoots, and flowers and about 15 cm tall from the water level were selected for the present experiment. By using pure culture technique (Spread plate technique) five types of rhizoplane microbes were selected on the basis of colony morphology. Isolates were plated on nutrient agar plates containing 1 mg l^{-1} of Cu ions. Two out of five isolates, were screened and used for further analysis, because the remaining three isolates showed slow growth in the nutrient medium. Biochemical identification at genus level was carried out [11] and the species of bacterial strains were conformed using microbial identification system (BD BBL Crystal identification system).

2.2. Stock copper solution

A stock solution of Cu ions (1,000 mg l^{-1}) was prepared by dissolving copper sulfate ($CuSO_4{\cdot}5H_2O$) in double distilled water and diluted to get the desired concentration with ddH_2O. The concentration of copper ions was measured using atomic absorption spectrophotometer (GBC 96-Phorton lamp) after the digestion of sample with HNO_3 and $HClO_3$.

2.3. Effects of copper concentration and pH on biosorption of Cu ions

The bacterial isolates were inoculated aseptically in nutrient broth supplemented with 50, 100, 150, 200, 250, 500, 750, 1,000, 1,250, and 1,500 mg l^{-1} of Cu ions and incubated in incubator shaker at 150 rpm for 24 h. After incubation, the cells were centrifuged (at 3,000 rpm for 15 min) and the supernatant was collected and digested with nitric acid and perchloric acid in the ratio 3:1. The digested sample was then incubated for one hour at room temperature. Now the solution was injected in AAS for the estimation of Cu ions. Maximum sorption was observed at 500 mg l^{-1} of Cu ions containing aqueous solution. Hence the above concentration was taken as an optimum initial concentration for further analysis of other parameters.

Effect of pH on biosorption of Cu ions was determined by adjusting the pH of the medium range from 5 to 9 using 0.1 N HCl and 0.1 N NaOH. 500 mg l^{-1} of copper ions was added to the medium after sterilization. The bacterial isolates (1 ml) were inoculated aseptically in the medium containing Cu ions. After incubation, both the bacterial isolates were centrifuged and acid digested as mentioned above and the solution was analyzed in AAS to determine the concentration of Cu ions.

2.4. Effects of temperature on biosorption of Cu ions

The bacterial isolates were inoculated aseptically in the aqueous solution containing 500 mg l^{-1} of copper ions and incubated in a shaker at 150 rpm for 24 h at various temperatures (25℃, 37℃, and 45℃). After incubation, the cells were digested with nitric and perchloric acids as mentioned above and further analyzed in AAS for the estimation of Cu ions.

2.5. Data analysis

The amount of absorbed Cu ions per unit biosorbent was obtained by using the following expression [12].

$$q = [C_0 - C_1 V]/M \tag{1}$$

where q is the amount of heavy metal absorbed onto the unit amount of adsorbents ($\mathrm{mg\,g^{-1}}$) and C_0 and C_1 are the concentrations of metal ions from the solution ($\mathrm{mg\,l^{-1}}$) before and after biosorption, respectively; V is the volume of the aqueous phase and M is the amount of the absorbent (g).

2.6. Kinetic modeling

2.6.1. The pseudo-first-order equation

The pseudo-first-order equation [13] is generally expressed as follows,

$$dq_t/dt = k_1(q_{eq} - q_t) \tag{2}$$

where k_1 is the rate constant of pseudo-first-order biosorption ($\mathrm{min^{-1}}$) and q_{eq} and q_t denote the amount of biosorption equilibrium at time t ($\mathrm{mg\,g^{-1}}$), respectively. After integrating by applying boundary conditions, $q_t = 0$ at $t = 0$ and $q_t = q_\mathrm{t}$ at $t = t$, gives:

$$\log(q_{eq}/q_{eq} - q_t) = (k_1 t)/2.303 \tag{3}$$

Eq. (3) can be rearranged to obtain a linear form.

$$\log(q_{eq} - q_t) = \log q_{eq} - (k_1 t)/2.303 \tag{4}$$

A plot of log ($q_{eq} - q_t$) against t should give a straight line to confirm the applicability of the kinetic model. In a true first-order possess q_{eq} should be equal to the intercept of a plot of log ($q_{eq} - q_t$) against t.

2.6.2. The pseudo-second-order equation

If the rate of sorption is a second-order mechanism, pseudo-second-order chemisorption kinetics rate equation [14] is expressed as Eq. (4):

$$dq_t/dt = k_2(q_{eq} - q_t)^2 \tag{5}$$

where K_2 is the rate constant of pseudo-second-order absorption ($\mathrm{g^{-1}\,mg^{-1}\,min}$). For the boundary conditions $t = 0$ at $t = t$ and $q_\mathrm{t} = 0$ at $q_\mathrm{t} = q_\mathrm{t}$, integrated form of Eq. (5) becomes:

$$1/(q_{eq} - q_t) = 1/q_{eq} + K_2 t \tag{6}$$

This is the integrated rate law for pseudo-second-order reaction. The above Eq. (6) is rearranged to obtain a linear form.

$$(t/q_t) = 1/(K_2 q^2 eq) + (1/q_{eq})t \tag{7}$$

A plot of t/q_t vs. t should give linear relationship for the applicability of the second-order kinetics. The rate constant (K_2) and absorption at equilibrium (q_{eq}) is obtained from the intercept and slop, respectively.

2.6.3. Adsorption isotherms

The Langmuir model is described by the following equation:

$$q_e = \frac{q_{\max} \times b_{ce}}{1 + b_{ce}} \tag{8}$$

where C and q also shows residual metal concentration and the amount of metal absorbed on the adsorbent at equilibrium. The $k_\mathrm{d} = k_2/k_1$ is the Langmuir constant of the system. The semi-reciprocal plot of C/q vs. C was employed to generate the intercept k_d/q and the slope $1/q$.

The Freundlich equation is the empirical relationship for assumption of adsorption energy of a metal binding site on adsorbent [15,16]. The Freundlich model is described by,

$$q_e = K_F C_e^{1/n} \tag{9}$$

where q_e, metal uptake at equilibrium concentration $\mathrm{mg\,g^{-1}}$; C_e, equilibrium metal ion concentration, mg $\mathrm{g^{-1}}$; K_F, Freundlich's constant of adsorption capacity; and n, Freundlich's constant of adsorption intensity. The K_F was estimated from intercept and n from slope.

3. Results and discussion

3.1. Isolation and identification of rhizoplane bacterial isolates

Heavy metal resistant bacteria isolates were isolated from the root of water hyacinth and analyzed for their ability of biosorption of Cu ions from aqueous solution in batch systems. From this study, two types of pure culture of rhizoplane bacterial isolates showing different colony morphology (among 78 colonies) were isolated from root of water hyacinth. These two isolates were screened for their metal resistance on nutrient agar media containing various concentrations of Cu ions. The isolates were identified as *P. putida* and *B. cereus* using standard biochemical tests [11] and microbial identification system (BD BBL Crystal identification system) (Table 1).

Table 1
Biochemical characterization of bacterial isolates

Biochemical tests	Bacterial isolate—I	Bacterial isolate—II
Cell shape	Rod	Rod
Gram reaction	−	+
KOH reaction	+	+
Motility	+	+
Spore formation	−	+
Oxidase	+	−
Catalase	+	+
Lipase	+	+
Amylase	−	+
Pectinase	+	−
Cellulase	−	−
Urease	−	−
MacConkey agar	+	
Glucose fermentation	+A	+A
Sucrose	−	−
Maltose	−	+
Fructose	−	−
Lactose	−	−
KCN	+	−
H2S production	−	+
Citrate utilization	+	+
Identified species	*Pseudomonas* sp.	*Bacillus* sp.

Notes: +, positive; −, negative; +A, acid production.

3.2. Effects of initial concentration and contact time on biosorption of Cu ions

The time required for the biosorption of Cu ions on *P. putida* and *B. cereus* was studied at various initial concentrations (50, 100, 150, 200, 250, 500, 750, 1,000, 1,250, and 1,500 mg l^{-1}) at different intervals with 1 ml of biosorbent. Biosorption of Cu ions was estimated at various contact time (0, 4, 8, 12, 16, 20, and 24 h). Both the bacterial isolates were resistant to maximum concentration of 1,500 mg l^{-1} of Cu ions and found to decrease by further increasing concentration. One of the earlier study reported that *Pseudomonas* sp. is used for the removal of heavy metals like Cu, Cr, Cd, and Ni ions [17]. From their study, they reported that *P. putida* is specific for accumulation of Cu (II) ions. Another one study reported that *Bacillus* sp. have the ability to absorb Cu, Cd, and Pb ions [18].

Fig. 1(a) shows that the biosorption capacity sharply increases with increase in time and attains stability at 24 h after that the biosorption became marginal. Biosorption tendency of the tested biosorbent was increased with increase in initial concentration and found to reach saturated value at 1,500 mg l^{-1} of Cu ions. Fig. 1(b) explains the biosorption of Cu ions on *P. putida* and *B. cereus* was 856 and 800 mg l^{-1}, respectively. From this study, 84.8 and 83.8% of biosorption of Cu ions was noted at the initial concentration of 500 mg l^{-1} by *P. putida* and *B. cereus*, respectively. The observation of one of the previous studies shown that *Bacillus* sp. and *Pseudomonas* sp. absorbed 44.73 and 69.34% of Cu ions, respectively [18]. With the comparison of the previous studies, the bacterial isolates used in the present study were efficient for the removal of Cu ions from the contaminated aqueous solution.

3.3. Effects of pH on biosorption of Cu ions

Numerous studies have shown that pH is an important parameter for the biosorption of heavy metals using microbial biomass. The metal biosorption was depended onto the protonation and deprotonation of the carboxyl groups, which had pKa between 3 and 4 [19]. Biosorption of copper ions using *P. putida* and *B. cereus* was increased at pH 7 and gradually decreased at pH 9 (Fig. 1(c)). The amount of absorbed Cu ions from the initial concentration (500 mg l^{-1}) by *P. putida* and *B. cereus* was found to be 423 and 419 mg l^{-1}, respectively, at pH 7. *Pseudomonas* and *Bacillus* sp. has the ability to uptake maximum Cu ions at pH 7 [18]. The rate of biosorption was decreased at particular pH values due to the competitiveness of the oxyanion of Cu and OH^{-} ions in the bulk. These results suggested that pH affects the solubility of metals and ionization state of functional groups like carboxylate, phosphate, and amino groups of cell walls of the biosorbent [20]. As pH increases deprotonation starts and thereby results in decreased biosorption capacity [21].

3.4. Effects of temperature on biosorption of Cu ions

Biosorption of Cu ions on *P. putida* and *B. cereus* was increased at 37°C and gradually decreased at 45°C (Fig. 1(d)). Amount of biosorbed Cu ions by *P. putida* and *B. cereus* were found to be 420 and 415 mg l^{-1}, respectively, at 37°C with the initial concentration of 500 mg l^{-1}. In this study, biosorption equilibrium of Cu ions from *P. putida* and *B. cereus* was at 37°C. For uptake of heavy metals by *Pseudomonas mendocina* and *Cattleya luteola* 30°C is optimum [22]. Temperature effects are confined to metabolism-depended metal accumulation. The bacterial isolates isolated were mesophylic bacterial isolates which grow at 30–40°C. Beyond this range, the metabolic activities of the isolates were repaired and also the metal biosorption ability was reduced.

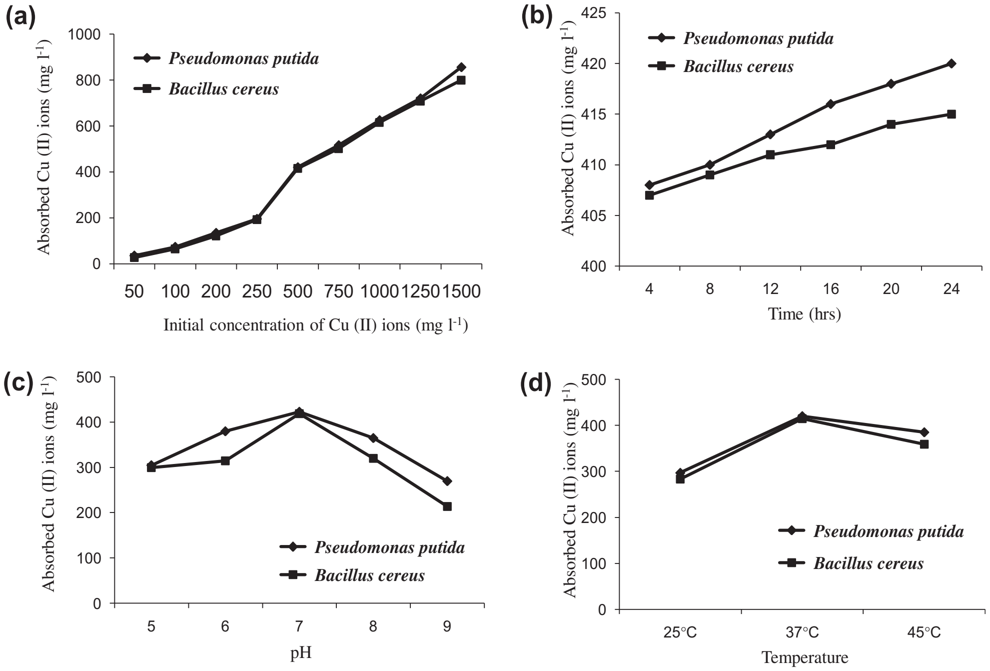

Fig. 1. Biosorption of Cu ions by *P. putida* and *B. cereus* (a) at various initial concentrations of Cu ions, (b) at different time interval with 500 mg l^{-1} of Cu ions, (c) at various pH and (d) at different temperature.

3.5. Biosorption of kinetic modeling

The kinetics for biosorption of Cu ions on bacterial isolates were analyzed using different kinetic models, these include, the pseudo-first-order [13] and pseudo-second-order [14]. The experimental biosorption capacities and theoretical values for *P. putida* and *B. cereus* were compared by first- and second-order rate equations (Table 2). The theoretical q_{eq} values, estimated from the first-order kinetics, gave different values when compared to experimental values and the correlation coefficients, were found to be slightly lower. The correlation coefficients (R^2) of both the models were greater than 0.9, indicating that second-order adequately describes the data acquired in metal biosorption assays (Fig. 2(a) and (b)). The pseudo-second-order equation fit for the biosorption of heavy metals [23]. These results suggested second-order mechanism for biosorption of Cu ions by *P. putida* and *B. cereus* was predominant. The results showed that biosorption systems were best described through pseudo-second-order kinetic model than pseudo-first-order kinetic model. The study of sorption kinetics describes uptake of biosorbent rate and controls the residence time of biosorbate at the solid–liquid interface [12]. The agreement between the experimental data and the predicted values for each model was expressed by the coefficient of determination (r^2). A relatively high r^2 value indicates the model, so examined, successfully describes the kinetics of metal ion biosorption onto the bacterial isolates.

3.6. Langmuir and Freundlich isotherms

Fig. 2(c) showed the Langmuir plot for the adsorption of Cu ions on *P. putida* and *B. cereus* biomass. The Langmuir constants (q_m and k_d) along with correlation

Table 2
The pseudo-first and second-order kinetics constant for biosorption of Cu ions on *P. putida* and *B. cereus*

Biosorbents	Experimental q_{ex} (mg g^{-1})	Pseudo-first-order			Pseudo-second-order		
		q_{eq} (mg g^{-1})	$K_1 \times 10^{-2}$ (min^{-1})	R^2	q_e (mg g^{-1})	$K_2 \times 10^0$ (min^{-1})	R^2
P. putida	423	26	4.388	0.9652	417	8	1
B. cereus	419	21	6.609	0.9496	417	4.8	0.9999

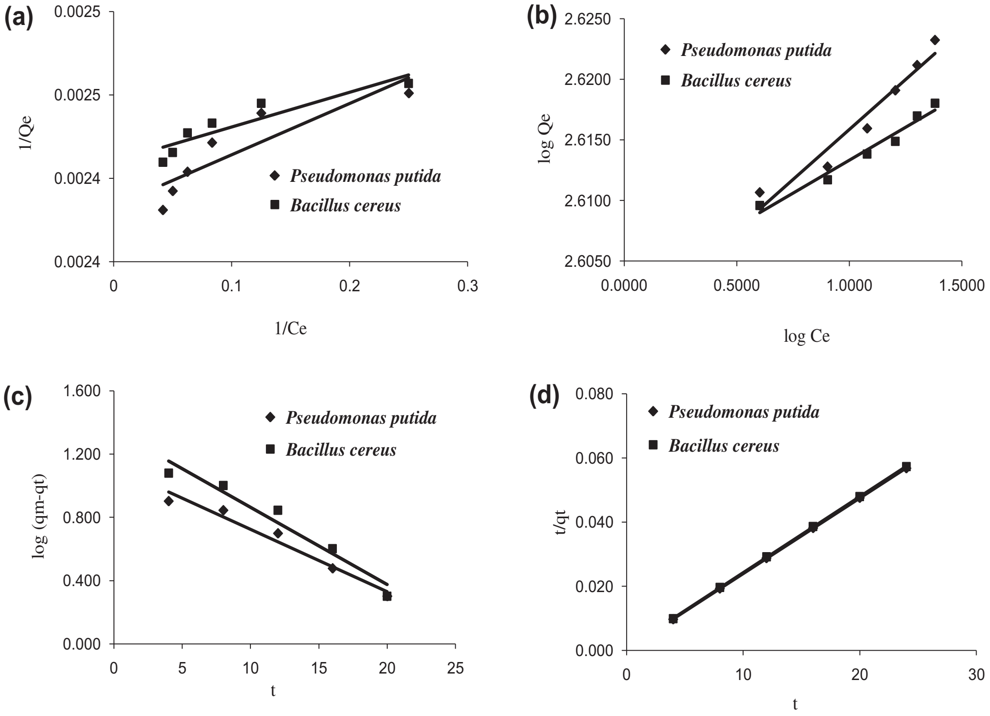

Fig. 2. (a) The pseudo-first-order kinetics constant log ($q_m - q_t$) plot on biosorption of Cu ions, (b) pseudo-second-order kinetics constant t/q_t on biosorption of Cu ions, (c) langmuir adsorption isotherms of Cu ions on the biosorption and (d) freundlich plot of biosorption of Cu ions on *P. putida* and *B. cereus* at pH—7 from aqueous solution. Initial concentration —500 mg l^{-1}; biomass concentration—1 ml.

coefficients (R^2) were calculated and the results were given in Table 3. The maximum capacity (q_m) determined from Langmuir isotherm, defined the total capacity of the biosorbents for Cu ions. The order of maximum capacity (q_m) for the biosorbents of Cu removal were found to be *P. putida* > *B. cereus* (Table 3). It is clear that the increase of q_m value was due to adsorptive sites on biosorbents. The presence of small k_d value indicated that the metal ions had high binding affinity for the biosorbent and k_d values were presented in Table 3. The k_d values for the absorption of Cu ions were 8 and 12 for *P. putida* and *B. cereus,* respectively. This inferred that *P. putida* shows greater absorption capacity than *B. cereus*. The adsorption isotherm model was used to characterize the interaction of Cu ions with the bacterial biomass. The Langmuir model was based on the assumption that maximum adsorption occurs when a saturated monolayer of solute molecules is present on the adsorbent surface, the energy of adsorption is constant, and there is no migration of adsorbate molecules in the surface plane [24,25].

Freundlich constants k_F and n showed easy separation of metal ions from aqueous medium and indicated favorable adsorption [26]. Fig. 2(d) shows the Freundlich plot for the adsorption of Cu ions by *P. putida* and *B. cereus* with intercept k_F (0.414 for *P. putida* and 0.415 for *B. cereus*) as the adsorption capacity of the adsorbents with slope $1/n$ (60.6 for *P. putida* and 91.7 for *B. cereus*) as the effect of

Table 3
Langmuir and Freundlich isotherm model constant and correlation co efficient for biosorption of Cu ions on *P. putida* and *B. cereus*

Biosorbents	Experimental $q_{eq,ex}$ (mg g^{-1})	Langmuir constant			Freundlich constant		
		q_m (mg g^{-1})	$K_d \times 10^0$ (M)	R^2	K_F	N	R^2
P. putida	420	417	8	0.782	0.414	60.6	0.9423
B. cereus	415	417	12	0.8348	0.415	91.7	0.9664

concentration of the adsorption capacity of *P. putida* and *B. cereus* in aqueous solution (Table 3). The *n* values showed easy uptake of Cu ions from aqueous solution with high adsorption capacity. In these systems, the metal removal process was based on solid–liquid contacting and separation process. In the present investigation, Freundlich model and Langmuir model fitted well for the biosorption of Cu ions from aqueous solution by the bacterial isolates.

4. Conclusion

The major problem in all industries is the treatment of their effluents. Several investigations were made for the effluent treatment. The present investigation deals with the isolation of two rhizoplane bacterial isolates identifies as *P. putida* and *B. cereus* from water hyacinth and their biosorption ability of Cu ions present in aqueous medium. The biosorption rate was optimized by providing various physiological parameters like pH and temperature. The highest biosorption rate was observed at pH 7 and temperature of 37˚C for both isolates. Pseudo-second-order kinetics model fitted well for the biosorption of Cu ions on bacterial isolates and at the same time Freundlich isotherm fitted well than Langmuir model. The result of the study implies that the two bacteria isolated from the rhizoplane of weeds may have a potential as bioremediation agents to remove Cu ions from the effluents of various industries. In addition to this, the method followed here is eco-friendly in nature and economical than the conventional methods. Also after the treatment with biological matters, effluents consist of permissible level of copper ions. Thus, the treated water will be utilized for the purpose of irrigation which never causes toxic effects on human beings.

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