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SORPTION POTENTIAL OF *PENICILLIUM JANTHINELLUM* FOR REMOVAL OF Cu(II) FROM ELECTROPLATING INDUSTRIAL EFFLUENT

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ABSTRACT

To study the extent of removal of Cu(II) ions from the electroplating industrial effluent, metal resistant fungal strain of *Penicillium janthinellum* was selected for the present study. Response surface methodology was used to optimize the sorption process variables i.e. temperature (20-40 ºC), biomass concentration (0.5-2.5 mg/l), pH (2.0-6.0) and initial concentration of metal ions (20-100 mg/l). The maximum removal 59.48% of Cu (II) ions was observed at temperature 33.7 °C, biomass concentration 1.65 g/L, pH 5.88, and metal ions concentration 60.12 mg/L. Experimental results shows that sorption of Cu (II) ions by *P. janthinellum* indicates good biosorbent for removal of heavy metals from aqueous solution. However, the Cu(II) ions removal efficiency by *P. janthinellum* in electroplating industrial effluent was found to be 35%.

KEYWORDS: **Sorption, Heavy Metals, Response Surface Methodology, *Penicillium janthinellum*, Electroplating Industrial Effluent.**

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INTRODUCTION

It is well known that there is a permissible limit of each metal, above which they are toxic and even hazardous. Heavy metals like Cd, Cu, Co, Hg, Cr, Fe, Se, As, Ni, Zn, Pb etc. are the most toxic and hazardous pollutants; often occur in industrial effluent at significant concentration. They can cause serious damage to biota and the environment, because they are accumulated through trophic chain and produce toxic effects and teratogenic changes in plants, animals and human beings, they also persist in sediments and released into water bodies [1,2]. These toxic metals can causes accumulative poisoning, cancer and brain damage when found above tolerance levels[3]. Now-a-days primary concern about heavy metals pollution is due to their potential toxicity, persistence and tendency to become concentrated in food chain [4-6]. Long term exposure to copper can cause irritation of the nose, mouth and eyes and it causes headaches, stomach-aches, dizziness, vomiting and diarrhoea. Exposure to copper fumes, dusts, or mists may result in fever with atrophic changes in the nasal mucous membranes. Chronic copper poisoning results in Wilson's disease, characterized by hepatic cirrhosis, brain damage, demyelination, renal disease, and copper deposition in the cornea[7].

Strict environmental protection legislation and public environmental concern is helpful for the human beings to explore novel techniques for treatment of toxic metals in the industrial effluent[8]. Development in the field of environmental biotechnology indicates that bacteria, fungi, yeast, algae can remove heavy metals from aqueous solution. Many researchers have focused their efforts upon heavy metal accumulation within the body of living microorganisms[9]. The use of these microbial biosorbents for removal of toxic heavy metals from aqueous solution has emerged as an alternative to existing methods as a result of the search for low cost innovative methods[10]. The uptake of heavy metal ions can take place by entrapment in cellular structure and subsequent sorption into binding sites present in the cellular structure. Aksu (2002)[11] reported that the use of dead microbial cells in biosorption is more advantageous for water treatment because they are not affected by toxic waste, and did not require a continuous supply of nutrients and can be regenerated and reuse for many cycles Volesky (1986)[12] also noticed that dead biomass possess high metal sorbing potential. Recently a number of reports have focused on the use of microbial-based biosorbents such as yeast, bacteria, algae and fungi[13-15].

In the present investigation metal resistant fungal strain of *P. janthinellum* have been used as biosorbent, to study its potential for sorption of Cu(II) ions from synthetic as well as electroplating industrial effluent.

MATERIALS AND METHODS

(i) Preparation of Synthetic Copper Solution

3.929 gm of copper sulphate ($CuSO_4.5H_2O$) was dissolved in 1 L double distilled water to prepare 1000 ppm stock solution. This solution contains divalent copper. A working solution was further prepared by diluting the stock solution.

(ii) Culture Medium Preparation and Biomass Genesis

The culture medium was prepared accordingly to kapoor et al. (1999)[16]. The dead biomass of *P. janthinellum* was prepared by giving autoclave treatment (15lb, 120 ºC for 20 to 25 minutes) to culture biomass. After that, the fungal biomass was dried at 60 ºC for a period of 16-24 hours and powered to obtain particles of size upto 0.3 mm. The powered biomass was stored in the zip load polyethylene bags, for the sorption experiments.

(iii) Biosorption Assay

A general biosorption assay was designed for testing biomass retention of heavy metals in non growth conditions. The 1000ppm $CuSO_4$

stock solution was prepared in double distilled water. The stock solution was diluted with double distilled water to obtain the desired concentration range of Cu(II) solutions. The pH values were varied from 2-6 to avoid possible metal hydroxide precipitation. The metal solution pH was adjusted by using 0.1 M HCl or NaOH. The chemicals used were of analytical reagent grade. For biosorption purpose, 100ml biosorption mixture was taken as in the experimental design. Suspensions were filtered through Whatman filter paper no.1 to obtain biomass free supernatants. The Cu(II) ions concentration in supernatants were determined in duplicates and their decrease was estimated by comparison to the biomass free control biosorption mixture.

(iv) Analytical Procedures

The concentration of Cu (II) ions in the test solution was determined by using atomic absorption spectrophotometer (GBC-932 Plus). Biosorption efficiency was calculated using following equation 1.

$$\% \text{ Removal} = \frac{(C_0 - C_e)}{C_0} \text{X } 100 \quad ....1$$

Where, Co is the initial metal ions concentration before equilibrium and Ce is the metals ions concentration after 1 h equilibrium period.

(v) Establishment of Response Surface Methodology (RSM)

Box-Behnken design model, which is the standard response surface methodology (RSM), was established on the basis of Design Expert software (Stat Ease, 8.0.7.1 trial version) for the optimization of the biosorption process. The experimental design, four independent variables i.e. pH (2.0-6.0), temperature (20-40 ^oC), adsorbent dose (0.5-2.5 g/L), initial metal ion concentration (20-100 mg/L) were taken and shaken for 1hour to obtain response i.e. biosorption of Cu (II) ions. For optimization of all the parameters experiments were conducted accordingly RSM experimental design taking 100 ml of metal solution in 250 ml Erlenmyer flask by keeping the time constant i.e. for 60 min. The experimental design was applied after selection range of each variable (maximum and minimum) as shown in Table 1. The Box Behnken design contained a total of 29 experiments with the 24 experiments organized in a factorial design with the 5 experimental trials involving the replication of the central point. Repeated observations at the center point were used to estimate the experimental error employed. The obtained response and run experiments were shown in Table 2

(vi) Statistical Analysis

The quadratic equation model for predicting the optimal point was expressed according to equations (2). Four parameters and 15 coefficients were estimated, i.e. the coefficients for the four main effects, four quadratic effects, six interactions and one constant[17]. RSM makes it possible to represent independent process parameters in quantitative form Box and Behnken (1960)[18] as:

$$Y = b_0 + \sum b_i X_i \sum b_{ii} X_i^2 + \sum b_{ij} X_i X_j \ .... \ (2)$$

Where, Y is predicted response, b_o is the response function and X_i, X_j, b_i, and b_j are experimental coded variables respectively (Table 1). These coded variables are related to uncoded variables using the following relation eq. (3).

$$Xi = \frac{2(a_i - b_i)}{d_i} \ .... \ (3)$$

Where a_i is the variable value in actual units of the $_i$th observation, b_i is the mean of highest and lowest variable value of a_i and d_i is the difference between the highest and the lowest variable value of a_i. The variables given table 1. is based on above relationship in equation (3). The overall second - order polynomial mathematical relationship of the response Y and the four variables i.e. temperature, Biomass concentration, pH and metal ions concentration can be approximated by the quadratic equation (4). Total 29 experiments are design as given in table 2.

Table 1
Process variables and their levels

Independent variables	Symbols		Level	
	Coded	Uncoded	Coded	Uncoded
Temperature (^oC)	X_1	A	1	40
			0	30
			-1	20
Adsorbent dose (g/l)	X_2	B	1	2.5
			0	1.5
			-1	0.5
pH	X_3	C	1	6
			0	4
			-1	2
Metal ions concentration (ppm)	X_4	D	1	100
			0	60
			-1	20

(vii) Physico-chemical Characteristics of Electroplating Wastewater

Characteristics of the electroplating industrial effluent of Yamunanagar, Haryana are shown in Table 4. Analytical Reagent Grade (AR) chemicals were used throughout the study without any further purification. To prepare all the reagents and calibration standards, double glass distilled water was used. The glassware were washed with dilute nitric acid (1.15) followed by several portions of distilled water. The pH of the samples was measured as soon as possible after collection. All the parameters were analyzed according to APHA (2003)[19], standard methods. All the experiments were carried out in triplicate. The results were reproducible within ±3% error limit.

RESULTS AND DISCUSSION

Sorption study was carried out by using dead fungal biomass of *P. janthinellum*. The present investigation deals with the removal of heavy metals from synthetic solution as well as electroplating industrial effluent. Batch study and response surface methodology (RSM) were made to optimize the process variables for effective removal of Cu (II) from synthetic solution as well as electroplating industrial effluent. The results obtained from laboratory study of metals on biosorption are discussed in the following sections.

1. Metal Tolerance Assay for Screening of Fungal Strains

The metal resistant strains were screened by enrichment culture techniques on agar plates amended with 10 mg/L of Cu(II) and identified as *P. janthinellum* on the basis of morphological and physiological characteristics.

2. Validation of Response Surface Models and Statistical Analysis

The second order polynomial equation was used to find out the relationship between independent variables and response. The regression equation coefficients were calculated and data were fitted to a second-order polynomial equation for removal of Cu(II) with *P. janthinellum (Eq. 3 and 4)*. The analysis of variance (ANOVA) for sorption of Cu(II) ions with fungal biomass was used in order to ensure a good model, the test for significance of regression model and the results of ANOVA are in Table 2 and 3. Prob > F less than 0.0500 indicate model terms are significant and value greater than 0.05 indicate the non significant terms. The non significant value lack of fit 0.0593 for Cu (II) for *P. janthinellum* shows that quadratic model is valid for present study. The predicted R^2 and adjusted R^2 0.9974 and 0.9990 of Cu (II) for *P. janthinellum* indicate reasonable agreement with the values of R^2, which is closer to 1.0, indicates the better fitness of model in the experimental data.

Table 2
Experimental design and results of the box behnken model

Std	Temperature (ºC)	Adsorbent Dose (g/l)	pH	Metal Ions Conc. (ppm)	*P. janthinellum* (% Removal)
					(Cu)
1	-1	-1	0	0	39.2
2	1	-1	0	0	36.4
3	-1	1	0	0	56.6
4	1	1	0	0	52.8
5	0	0	-1	-1	11.2
6	0	0	1	-1	54.3
7	0	0	-1	1	09.2
8	0	0	1	1	53.4
9	-1	0	0	-1	52.9
10	1	0	0	-1	50.5
11	-1	0	0	1	53.4
12	1	0	0	1	48.2
13	0	-1	-1	0	0.4
14	0	1	-1	0	08.3
15	0	-1	1	0	33.2
16	0	1	1	0	61.5
17	-1	0	-1	0	09.8
18	1	0	-1	0	08.5
19	-1	0	1	0	56.6
20	1	0	1	0	49.5
21	0	-1	0	-1	40.5
22	0	1	0	-1	54.5
23	0	-1	0	1	34.5
24	0	1	0	1	55.7
25*	0	0	0	0	50.5
26*	0	0	0	0	50.4
27*	0	0	0	0	51.1
28*	0	0	0	0	50.8
29*	0	0	0	0	50.6

** Central value*

% Cu (II) Removal (P. janthinellum) = + 50.68 - 1.89 * A + 8.77 * B + 21.75 * C-0.80 * D - 0.25 *A * B - 1.45 * A * C - 0.71 * A * D + 5.10* B* C + 1.80 * B * D+0.26*C*D+0.16*A²-4.93 *B² -19.65* C² +0.64*D²……………………………………(4)

$$\%\,Cu\,(II)\ Removal = +50.68 - 1.89 A + 8.77 B + 21.75 C - 0.80 D - 0.25 AB - 1.45 AC - 0.71 AD + 5.10 BC + 1.80 BD + 0.26 CD + 0.16 A^2 - 4.93 B^2 - 19.65 C^2 + 0.64 D^2 \quad (4)$$

Table 3
Analysis of variance (ANOVA) for quadratic model of P. janthinellum for Cu (II)

Source	Sum of Squares	df	Mean Square	F- Value	p-value Prob > F	
Model	9521.08	14	680.07	2133.39	< 0.0001	significant
A-Temperature	42.71	1	42.714	133.99	< 0.0001	
B-Adsobent	922.25	1	922.25	2893.10	< 0.0001	
C-pH	5678.92	1	5678.92	17814.74	< 0.0001	
D-Metal Ions	7.66	1	7.664	24.04	0.0002	
AB	0.25	1	0.25	0.784	0.3908	
AC	8.41	1	8.41	26.38	0.0002	
AD	2.016	1	2.016	6.325	0.0247	
BC	104.04	1	104.04	326.37	< 0.0001	
BD	12.96	1	12.96	40.655	< 0.0001	
CD	0.275	1	0.275	0.8646	0.3682	
A^2	0.172	1	0.172	0.5400	0.4745	
B^2	157.78	1	157.78	494.97	< 0.0001	
C^2	2504.79	1	2504.79	7857.50	< 0.0001	
D^2	2.69	1	2.691	8.443	0.0115	
Residual	4.46	14	0.318			
Lack of Fit	4.15	10	0.415	5.395	0.0593	Not significant
Pure Error	0.308	4	0.077			
Cor Total	9525.55	28				
Std. Dev.	0.564					
Mean	40.84					
C.V. %	1.38					
PRESS	24.41					
R-Squared	0.9995					
Adj R-Squared	0.9990					
Pred R-Squared	0.9974					

C.V. = Coefficient of variance

High value of coefficient of pH parameter estimate for linear and quadratic terms than other variables showing a high level of significance indicate the most important variable in the sorption process (Eq. 4). The minimum value of standard error i.e. 0.20 for Cu (II) around the centeriod also validates the suitability of the model for the present study shown in fig. 1.

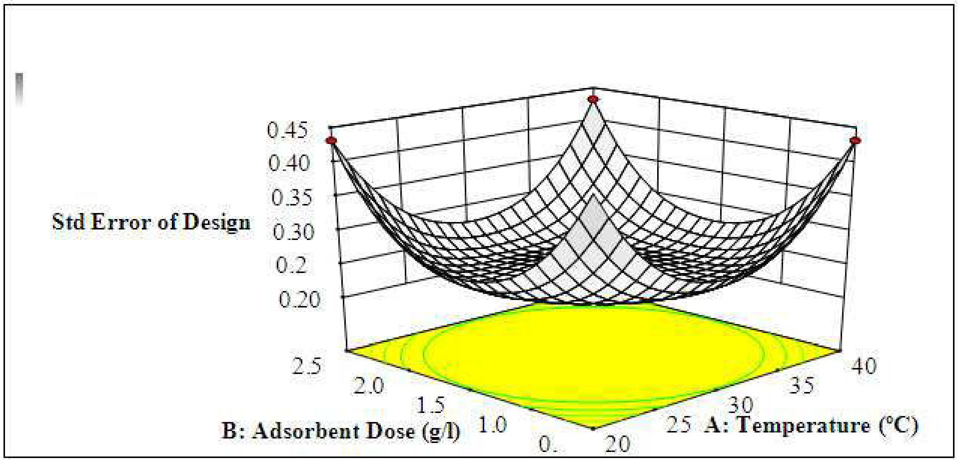

Figure 1
Standard error design of the model for Cu(II)

3. Interactive Effect of Variables for the Sorption Study of Metal Ions

On the basis of quadratic polynomial equation of response surface methodology (Eq. 3 and 4), the effect of independent variables i.e. temperature, adsorbent dose, pH, and initial metal ions concentration for sorption were analyzed.

4. Interactive Effect of pH and Temperature on Sorption of Metal Ions Holding Other Variable at Central Value

Interactive effect of pH and temperature on sorption of Cu (II) has shown in fig. 2 for *P. janthinellum*, holding other variable at central value. The 3D response surface plot shows the interactive effects of the pH and temperature the most important variables on the biosorption study of the heavy metal ions by keeping the other variables at fixed value. The significant value i.e. Prob > F lesser than 0.05 for both the metal ions indicate that it is the most important variable effecting the sorption of the heavy metals. This is also supported by the higher value of the linear coefficient for the variables (Eq. 4). The negative value of temperature coefficient for the variables (pH and temperature) of Cu(II) indicates the existence of the optimum value within the assay range[20]. As pH increase, the number of negatively charged sites increase and the number of positively charged sites decrease. A negatively charged surface site on adsorbent does not favour the adsorption of anions due to electrostatic repulsion[21]. As the pH increase, the metal sorption capacity increase significantly. However, with the restriction of forming insoluble hydroxide at high pH, the optimum operating pH was 5.88 for biosorption of Cu(II). The metal binding mechanism seems to involve the release of protons into solution, to some extent, as biosorption proceeds. As the pH was lowered, the overall surface charge of cell surface will become positive, whereas at higher pH values the overall surface charge will become negative, resulting in an increase of metals cationic biosorption[22]. At the lower pH more of the binding sites are occupied by the H^+ ions which compete for the metal ions but as the pH increases the H^+ ions decreases the sorption of the heavy metal increases. However, the ion exchange is not the principle mechanism.

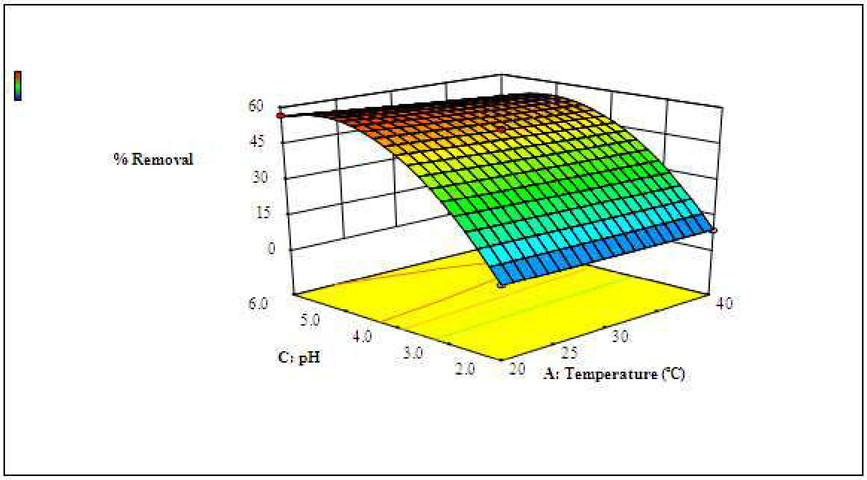

Figure 2
Interactive effect of pH (C) and temperature (A) on sorption of metal ions holding other variable at central value by P. janthinellum.

The medium pH affects solubility of metals and ionization state of functional groups like carboxylate, phosphate, methyl and amino groups of cell wall. The carboxylate and phosphate groups carry negative charges that allow the components of the cell wall to be potent scavengers of cations. When pH increases, adsorbent surface were more negatively charged with subsequent attraction of metallic ions with a positive charge and sorption process was favoured until a maximum was reached.

5. Interactive Effect of pH and Adsorbent Dose on Sorption of Metal Ions

The significant value of quadratic coefficient indicate that low and high pH are about the same but there is a better intermediate pH also more widely spaced contours in interactive three dimensional graph of pH indicated that it is the most important parameter effecting the sorption of the metal ions. The Interactive effect of pH and adsorbent on biosorption of Cu(II) shown in fig. 3 by *P. janthinellum*, holding other variable (Temperature and metal ions concentration) at central value. The positive value of the interactive coefficient indicated the significance of the variable interaction. The larger value of the linear coefficient for adsorbent dose indicated that it is the second most important variable after pH (Eq. 4). The negative and insignificant value for sorbent dose indicated the existence of the optimum beyond that no much affect on sorption with higher adsorbent dose.

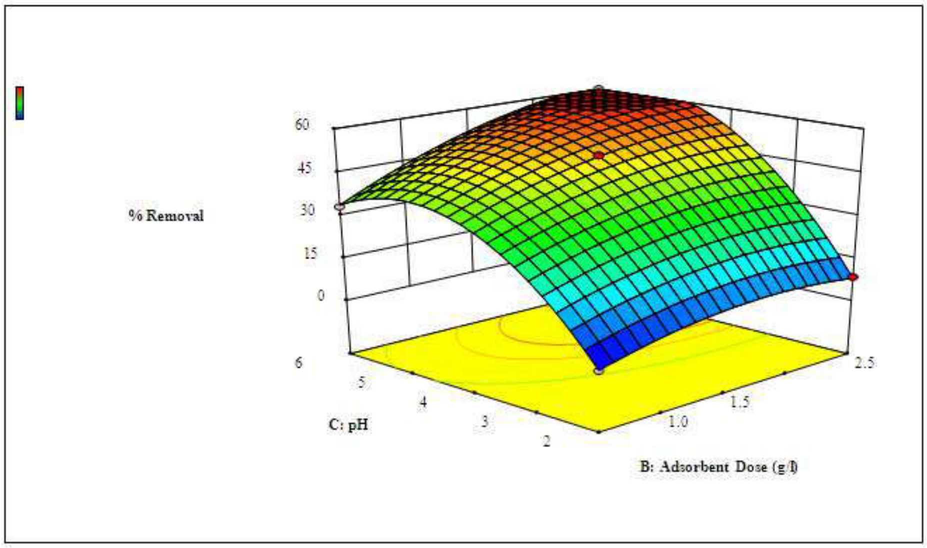

Figure 3
Interactive effect of pH (C) and adsorbent dose (B) on sorption of metal ions holding other variable at central value by Penicillium janthinellum.

6. Interactive Effect of Metal Ions Concentration and pH on Sorption of Metal Ions

The interactive effect of the metal ions concentration and pH on sorption of metal ions is observed by holding the temperature and adsorbent dose 30°C and 1.5 g/L respectively at central value. The significant value of probability <F for Cu(II) as a result of interactive effect metal ions and pH (Table 2 and 3) indicates the significant terms for sorption by *P. janthinellum*. Interactive effect of metal ions concentration and pH on sorption of Cu(II) shown in fig. 4 by *P. janthinellum*.

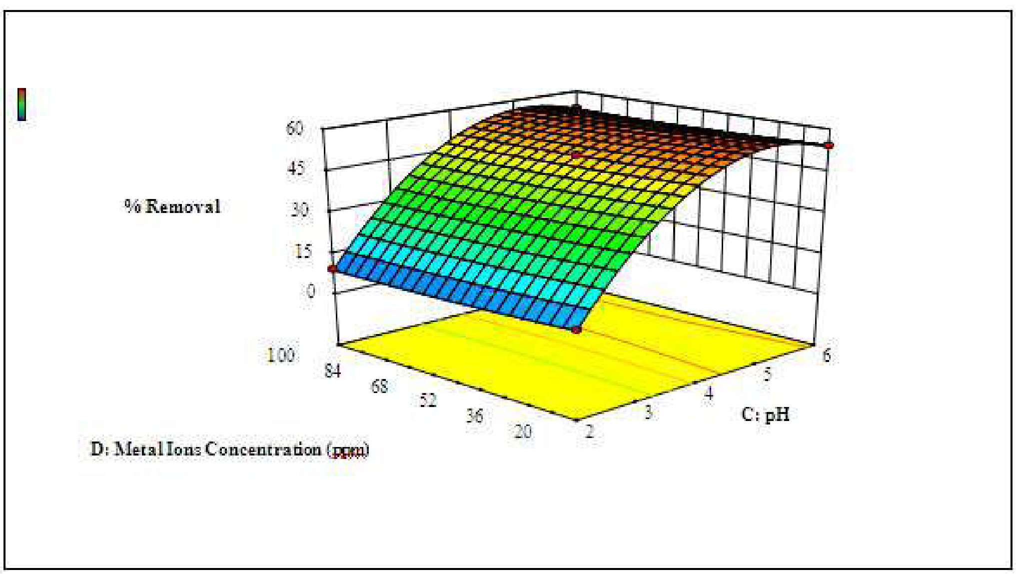

Figure 4
Interactive effect of metal dose (D) and pH (C) on sorption of metal ions holding other variable at central value by P. janthinellum.

7. Removal of Cu (II) Ions by P. janthinellum from Electroplating Industrial Effluent

The removal of Cu(II) ions from electroplating industrial wastewater by dead biomass of *P. janthinellum* was studied. The Cu(II) removal efficiency of *P. janthinellum* from electroplating industrial effluent at optimized conditions was found to be 35% which was lower than the synthetic aqueous solution of Cu(II). This may be due to the presence of various other heavy metals (Table 4). However, Recently Issac et al (2012)[23] have reported 90.3% Cu removal from electroplating effluent using dry *Aspergillus oryzae* biomass.

Table 4
Characteristics of the electroplating wastewater

Parameters	**Electroplating Industrial Effluent of Yamunanagar, Haryana**
Colour	Yellowish green
Total Dissolved Solids	13055
Total Suspended solids	28.5
pH	2.35
BOD	104.5
COD	510
Sulphate	369
Phosphate	5.8
Pb(II)	5.8
Cu(II)	35.4
Zn(II)	32.1
Total iron	14.8
Ni(II)	211
CN	---
Cr(VI)	32.8
Cd(II)	20.5

**** All units in mg/L except pH***

CONCLUSION

Three dimensional response plots indicate that the sorption process varies with other process variables i.e. temperature, adsorbent dose, pH, metal ions concentration. The significant coefficient for the term pH indicated that it the most important environmental parameter affecting the biosorption process. The maximum removal 59.48% of Cu(II) was observed at temperature 33.7°C, biomass concentration 1.65 g/L, pH 5.88, and metal ions concentration 60.12 mg/L and which is very close to the predicted value of model. To make the sorption process more economical it is necessary to regenerate the biosorbent for reusing in multiple sorption cycles. The present result of this study shows that *P. janthinellum* is a good biosorbent for removal of Cu(II) ions from aqueous solution and in electroplating industrial effluent the copper removal efficiency by *P. janthinellum* was found to be 35%. Therefore it is suggested that a complete experiment should be performed on the biosorbent of *Penicillium janthinellum* for sorption of other metal ions from aqueous as well as electroplating industrial wastewater.

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