

Optimization of Process Parameters for Cd (II) Biosorption onto a Cd (II) Resistant Yeast Strain *Candida tropicalis* XTA1874 in Aqueous Medium

Abstract: Biosorptive removal of Cd(II) depends on various physico-chemical and nutritional factors. The effects of various important factors on the Cd(II) biosorption process by the strain *Candida tropicalis* XTA 1874 has been optimized in our present study. Our study depicts significant impacts of the physico-chemical parameters on Cd(II) biosorption. The micronutrients employed in our study also showed significant effects. Among the carbon and nitrogen sources glucose and urea showed the most significant impact on Cd(II) biosorption by the strain.

Keywords: Biosorption micro nutrients, optimization cadmium *Candida tropicalis* XTA1874 pollution

Excessive industrialization often considered as a civilization hallmark is inviting a severe curse to our society¹. Heavy metal contamination in water bodies is a glaring problem nowadays². Such technological advancements are often inviting several adverse impacts in our environment. The ill processed industrial effluents often bear wastes that are carcinogenic and dampen other health processes as well². Presence of heavy metals in such unprocessed effluents is a glaring problem nowadays^{2, 3}).

Heavy metals include metal and metalloids having atomic density greater than 5g cm⁻³⁴. All the transition metals such as cadmium (Cd), chromium(Cr), iron (Fe), mercury (Hg), arsenic (As) and lead (Pb) are included under this term^{5,6}. Heavy metal pollutants are non-degradable and are quite impossible to be removed completely from the environment.

Cadmium is one such heavy metal pollutant and has no physiological role. It is tremendously toxic even in trace amounts⁷. It can invite multiple health hazards if being in taken in from contaminated environment. The worth

mentioning toxic effects are cadmium induced renal toxicity, hepatotoxicity, pneumotoxicity, reproductive and endocrine toxicity and carcinogenicity above all⁸. Cadmium pollution in groundwater is aggravating in various regions in our country^{9,10}. To mediate its immediate removal various physico-chemical means of remediation techniques have been practiced since a long time. The worth mentioning are chemical precipitation, coagulation-flocculation, ion exchange and a plethora of membrane mediated techniques¹¹. But many of these techniques suffer from severe drawbacks such as high operational cost, incomplete removal of the metals from dilute solutions and generating toxic wastes¹². Biological remediation techniques on the other hand are gaining a new attraction over these conventional techniques nowadays. Biosorption is a biological remediation technique that uses surface adsorption capacity of biological adsorbents¹³. Biosorption is influenced by a variety of physico-chemical factors such as pH, temperature, biosorbent dosage, contact time and a variety of nutritional conditions affecting microbial growth^{14,15}. The present study reports on the empirical optimization studies of the physical parameters such as pH, temperature (°C), age of inoculum (h), volume of medium (mL), volume of inoculum (mL) as well as the effects of various micro nutrients or trace element on the biosorption of Cd(II) by a Cd(II) resistant strain *Candida tropicalis* XTA 1874.

Materials and Methods: Biosorbent Preparation: Cd(II) resistant strain *Candida tropicalis* XTA1874 was developed from a *Candida tropicalis* strain isolated from Tolly Canal, beneath Karunamoyee Bridge, M.G. Road, Tollygunge Golf Club, Tollygunge, Kolkata, West Bengal-700040.

Effects of Physical Parameters: Effects of Initial pH: Effect of initial pH was evaluated on Cd(II) biosorption process by the strain by using 0.19-0.39 g using 100mL solution of 500ppm of Cd(II) dissolved in yeast extract peptone dextrose broth. Temperature was maintained at 27°C. The experiment was conducted within the pH range of 4-7 in digital pH meter (CL110-chemLine). The pH was adjusted using 0.1 (N) NaOH and 0.1 (N) HCl.

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Effect of Temperature: Effect of temperature on the Cd(II) biosorption by strain was evaluated by using a temperature range of 26-32°C using B.O.D. incubator (5-50°C). The biomass dosage used was 0.32-1.69 g using 100 mL solution of 500 ppm of Cd(II) dissolved in yeast extract peptone dextrose broth.

Effect of Age of Inoculum: The strain treated with Cd(II) was incubated with varying incubation times ranging from 24-72 hours. The biomass dosage used was 0.35-1.32 g using 100 mL media with 500ppm of Cd(II) dissolved was used in the study.

Effect of Volume of Medium: The strain treated with Cd(II) was incubated with different volume of Yeast Extract Peptone Dextrose (YPD) media ranging from 24-35 mL. The biomass dosage used was 0.35-0.71 g using 100 mL media with 500ppm of Cd(II) dissolved was used in the study.

Effect of Volume of Inoculum: The strain treated with Cd(II) was incubated with different inoculum volume (1-6% containing 8×10^6 cells/mL) in 100 mL of Yeast Extract Peptone Dextrose (YPD) media. The biomass dosage used was 0.52-1.05 g using 100 mL media with 500ppm of Cd(II) dissolved was used in the study.

Effects of Micronutrients: **Effect of K_2HPO_4 :** The strain treated with Cd(II) was incubated with different K_2HPO_4 concentrations (%) ranging from 0.08 to 0.3g in 100 mL of Yeast Extract Peptone Dextrose (YPD) media. The biomass dosage used was 1.8-2.8 mg/mL using 100 mL media with 500ppm of Cd(II) dissolved was used in the study.

Effect of KH_2PO_4 : The strain treated with Cd(II) was incubated with different KH_2PO_4 concentrations (%) ranging from 0.08 to 0.2g in 100 mL of Yeast Extract Peptone Dextrose (YPD) media. The biomass dosage used was 1.7-2.6 mg/mL using 100 mL media with 500ppm of Cd(II) dissolved was used in the study.

Effect of $MgSO_4$: The strain treated with Cd(II) was incubated with different $MgSO_4 \cdot 7H_2O$ concentrations (%) ranging from 0.01 to 0.04g in 100 mL of Yeast Extract Peptone Dextrose (YPD) media. The biomass dosage used was 2.0-2.2 mg/mL using 100 mL media with 500ppm of Cd(II) dissolved was used in the study.

Effect of $Na_2MoO_4 \cdot 2H_2O$: The strain treated with Cd(II) was incubated with different $Na_2MoO_4 \cdot 2H_2O$ concentrations (%) ranging from 0.1 to 0.5g in 100 mL of Yeast Extract Peptone Dextrose (YPD) media. The biomass dosage used was 1.4-3.8 mg/mL using 100 mL media with 500ppm of Cd(II) dissolved was used in the study.

Effect of $CoCl_2 \cdot 5H_2O$: The strain treated with Cd(II) was incubated with different $CoCl_2 \cdot 5H_2O$ concentrations (%) ranging from 0.1 to 0.5g in 100 mL of Yeast Extract Peptone Dextrose (YPD) media. The biomass dosage used was 2.8-3.6 mg/mL using 100 mL media with 500ppm of Cd(II) dissolved was used in the study.

Effect of $CuCl_2$: The strain treated with Cd(II) was incubated with different $CuCl_2$ concentrations (%) ranging from 0.1 to 0.5g in 100 mL of Yeast Extract Peptone Dextrose (YPD) media. The biomass dosage used was 2.8-3.8 mg/mL using 100 mL media with 500ppm of Cd(II) dissolved was used in the study.

Selection of Carbon / Nitrogen Source: In the due course of the biosorption study appropriate carbon and nitrogen sources effecting Cd(II) biosorption were selected with various carbon sources (glucose, fructose, lactose, maltose, arabinose, xylose, galactose, sodium citrate, sodium acetate, glycerol and mannose) and various nitrogen sources (urea, ammonium sulfate, sodium nitrate, ammonium chloride, ammonium carbonate, ammonium oxalate, ammonium citrate, diammonium hydrogen phosphate and ammonium nitrate) have been studied. The basal medium was selected in the process was: K_2HPO_4 , 0.1%, K_2HPO_4 , 0.1%, $MgSO_4 \cdot 7H_2O$, 0.025%. Various carbon and nitrogen sources were added to the basal medium. The medium was inoculated and incubated under shaking conditions (Remi RS-12 R) at 180 rpm at 30°C for 72 hours. After incubation period, centrifugation (Remi c24BL) (10000 rpm, 10 mins) was carried out for cell separation and Cd(II) was measured in the supernatant by Atomic Absorption Spectroscopy (Shimadzu AA-7000, Japan) ².

Equilibrium Biosorption Studies for Cd(II): Equilibrium biosorption experiments by the developed resistant strain were carried out using Cd(II) containing YEPD broth solutions using Cd(II) chloride ($CdCl_2 \cdot H_2O$, NICE) prepared in distilled water. The Cd(II) concentration in the solution ranged from 15-500ppm. Biosorption experiments were carried out by adding dry biomass (0.15 g) in 100 ml broth medium. The pH of the solution was maintained at 6.8. The reaction mixture was agitated at 100 rpm on a rotary shaker (REMI, RS-12R). After various time intervals of contact time ranging between 3-72 hours the biomass was separated by centrifugation using cold centrifuge (Remi c24BL). Cd(II) content in the supernatant was detected by atomic absorption spectroscopy (Shimadzu AA-7000, Japan). The amount of Cd(II) removal per unit mass of the biosorbent at equilibrium conditions was calculated by the following equation:

$$q_e = (C_o - C_e) \times V/m \quad (1)$$

q_e signifies the amount of Cd(II) uptake (mg of Cd(II) removed/g dry weight) at equilibrium, V signifies the sample volume(ml), C_o is the concentration (ppm) of Cd(II) initially added to the medium and C_e is the final Cd(II) concentration(ppm) remained in the medium after equilibrium and m is the weight in dry mass of the biosorbent (g).

The percent Cd(II) removed at equilibrium by biosorption was calculated by the equation:

$$\% \text{ Cd(II) removal} = (C_o - C_e / C_o) \times 100 \quad (2)$$

C_o signifies the initial Cd(II) concentration (ppm), C_e signifies the Cd(II) concentration (ppm) in the supernatant after centrifugation at equilibrium. The percent removal can also be considered as biosorption efficiency.

Studies by Using Adsorption Isotherms: The equilibrium adsorption capacity of Cd(II) was calculated by employing the Langmuir and Freundlich isotherms. Langmuir model describes the formation of monolayer adsorption on homogeneous surface. It is expressed as the equation as:

$$q_e = q_{\max} K_L C_e / (1 + K_L C_e) \quad (3)$$

The linearized version of the equation is represented by:

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

The values of $q_{\max}$ and K_L were determined based on the linear dependence of $1/q_e$ on $1/C_e$. The term $q_{\max}$ is defined as maximum adsorption capacity (mg/g_{d.w.}) and K_L is the Langmuir constant (L/mg). From Langmuir equation the value of a dimensionless constant called R_L was calculated by the following formula ³:

$$R_L = 1 / (1 + K_L C_o) \quad (5)$$

The Freundlich isotherm implies multilayer adsorption on heterogeneous surfaces and takes into account the interactions between the molecules. The equation is represented below:

$$q_e = K_f \times (C_e)^{1/n} \quad (6)$$

The straight line fitting gives the equation:

$$\text{Log } q_e = \text{Log } K_f + 1/n \text{ Log } C_e \quad (7)$$

K_f is the Freundlich constant characterizing maximum adsorption capacity (L/g). The term n determines the characteristics of adsorption. Both K_f and n were determined from the linear plot of $\text{Log } q_e$ vs $\text{Log } C_e$.

Biosorption Kinetics: Among the kinetic models described in the literature those that express the order of chemical reactions are highly considered, especially the

pseudo first order (Lagergren) and pseudo second order (Ho and McKay) models. Pseudo first order kinetic model has been applied by using the linearized equation

$$\text{Ln } (q_e - q) = \text{Ln } q_e - k_1 t \quad (8)$$

The values of q_e and k_1 parameters were obtained by linear regression method employing the plots of $\text{Ln } (q_e - q)$ versus t

Pseudo second order kinetic modelling has been applied by using the linearized form

$$t/q_t = t/q_e + 1/k_2 q_e^2 \quad (9)$$

The pseudo second order parameters q_e and k_2 were determined by plotting t/q against t .

Results and Discussion: Effects of Physical Parameters: *Effect of Initial pH:* The solution pH is one of the most important factors in controlling the efficacy of biosorption. The principal hindrance comes from the hydrogen ion concentration in metal ion biosorption in aqueous solution¹⁶. The data obtained in our sorption experiment with the strain show that biosorption increases with increasing pH because of the depletion of the obstacle from hydrogen ions and reaches optimum (48.2%) at pH 6.5 at a biomass dosage 0.58 mg/mL dry cell mass (Figure 1a). The biosorption efficacy decreases after the optimum pH most probably due to hydroxide precipitation ¹⁴.

Effect of Temperature: Temperature is also a major determinant in effecting biosorption efficacy. As has been observed in the graph with increasing temperature biosorption increases and reaches optimum (62.29%) at 27°C at a biomass dosage of 0.71mg/mL dry cell mass (Figure 1b). In another biosorption study with *Ascomyces nidosum* it was also observed that that biosorption increases by 50-70% with increasing temperature by 4-23°C ¹⁷.

Effect of Age of Inoculum: Biosorption also increases with inoculum age and reaches maximum (66.13%) at biomass dosage of 1.06 mg/mL when the age of the inoculum is 48 h. Cell age is a vital determining factor in affecting the biosorption of metal ions. Increasing biosorption is observed generally in early stages of growth¹⁸.

Effect of Volume of Medium: In the biosorption study by using different volume of media it has been observed that at 25 mL of YPD broth medium the Cd(II) removal increases 78.83 % at biomass dosage 0.2 mg/mL (Figure 1d). Ample amount of nutrients aid in organismal growth and proliferation which is enhanced by increasing the

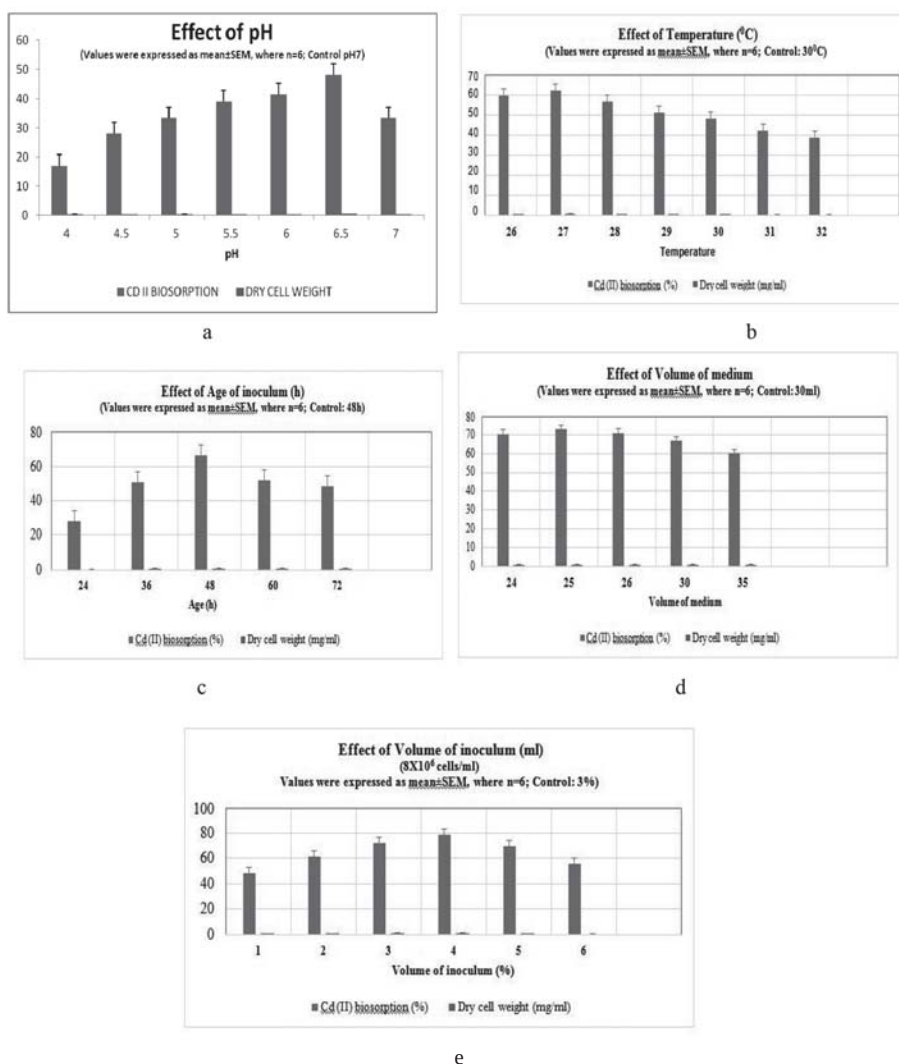


Figure 1: Effects of physico-chemical factors on Cd(II) biosorption by *Candida tropicalis* XTA1874. (a) pH (b) Temperature (c) Age of inoculum (d) Volume of medium (e) Volume of inoculum

volume of media. When it is increased too much the metal ions get diluted and do not properly access the available contact sites of the biosorbent.

Effect of Volume of Inoculum: The volume of inoculum is equivalent to the number of cells in the medium. Higher inoculum volume will produce higher cell count in the media. Higher cell number aids in metal ion biosorption providing ample amount of available contact sites on the biosorbent. On the contrary increasing the cell mass further diminishes metal ion biosorption. At higher biosorbent dosages the available contact sites get masked which may restrict the metal ion biosorption process owing to restricted access to the contact sites ¹⁴. In the present study we found maximum biosorption of 73.21% at volume of inoculum (8×10⁶ cells/mL) at biomass dosage of 0.71mg/mL dry cell mass.

Selection of Carbon and Nitrogen Source: The selection of proper carbon and nitrogen source is a quintessential step in governing microbial growth and its Cd(II) remediation capability. The evaluation of the importance of micronutrients has already been elucidated. However for efficient microbial growth and its bioremediation capability depends on optimization of media components focusing on the carbon and nitrogen sources. Among all the carbon sources tested in our study, the strain showed maximal biosorption (78.5%) of Cd(II) in glucose (450 mg/mL) at a biomass dosage of 2.1 mg/mL (Figure 2a). The other carbon sources showed biosorption in the following order: Fructose (38.3%) > Lactose (28.6%) > Maltose (22.1%) > Arabinose (17.1) > Xylose (11.3%) > Galactose (11%) > Sodium Citrate (9.8%) > Sodium Acetate (7.6%) > Glycerol (6.1%) > Mannose (5.4%) (Figure 3a). The bioremoval of Cd(II) by the strain was thereafter studied under various glucose concentrations from (270-540 mg/mL) and it was observed that maximum biosorption (78.5%) occurred under 450 mg/mL (10% C)

glucose concentrations and above this concentration biosorption decreased a little bit (Figure 2b). Among the various nitrogen sources selected for the present study, urea (10.4 mg/mL) showed maximum biosorption (78.5%) under the biomass dosage of 2.1 mg/mL (Figure 2c). During the study of the efficacy of various urea concentrations (7.8-26 mg/mL), 13 mg/mL (1% N) of urea showed maximum biosorption of 86.1% at a biomass dosage of 3.1 mg/mL (Figure 2d).

Effects of Micronutrients: K₂HPO₄ and KH₂PO₄ have important effects on the biosorption process. It has been observed that at 0.1% K₂HPO₄ and KH₂PO₄ concentrations biosorption increases maximum at 86.1% at dry cell mass of 2.7 mg/mL (Figure 3a, b). These two compounds actually act as buffer helping in maintaining the pH of the medium

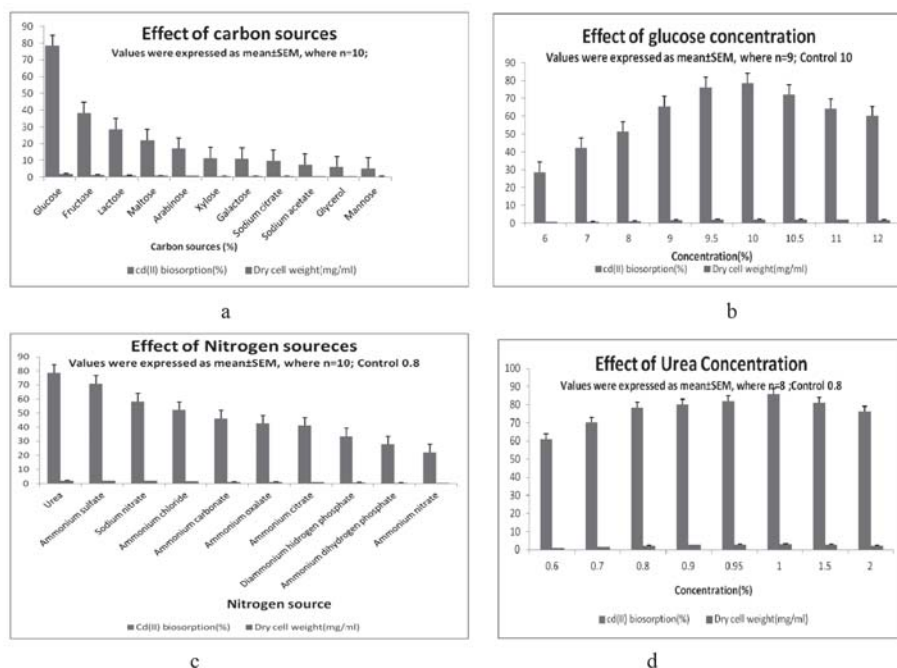


Figure 2. Effect of carbon and nitrogen sources on Cd(II) biosorption by *Candida tropicalis* XTA1874. (a)Carbon sources (b) Glucose concentration (c) Nitrogen sources(d) Urea concentration

(Q3)Table 1: Latest Trends in Cd(II) Biosorption

Biosorbent Used	Medium	Maximum Cd(II) Biosorption (%)	References
Freely suspended <i>Turbinaria ornata</i>	Aqueous solution	94.34%	25
Immobilized <i>Turbinaria ornata</i>		98.65%	
Mango seed Biosorbent	Aqueous solution	78%	26
<i>Weissella viridescens</i>	Vegetable and fruit juices	69.45-79.91%	27
ZY-6			
<i>Trichoderma</i> sp.	Aqueous solution	90.93%	28
<i>Chlorella vulgaris</i>	Aqueous solution	72%	29
<i>Coelastrella</i> sp.		82%	
Extracellular Polymeric substances (EPS) from <i>Agrobacterium tumefaciens</i> F2	Aqueous solution	94.41%	30
De-oiled palm carnal cake	Aqueous solution	99%	31
<i>Shewanella putrefaciens</i>	Liquid medium	86.54%	32
<i>Cassia fistula</i>	Aqueous solution	98%	33
<i>Chlorella vulgaris</i> (Live)	Aqueous solution	95.2%	34
<i>Chlorella vulgaris</i> (Dead)		96.8%	
<i>Ulva compressa</i>	Aqueous solution	94.8%	35
<i>Opuntia albicarpa</i>	Aqueous solution	53.3%	36
L. Scheinvar			
<i>Bacillus laterosporus</i>	Aqueous solution	85.47%	37
<i>Anabaena sphaerica</i>	Aqueous solution	84.5%	38
<i>Candida tropicalis</i>	Liquid medium	70%	39
CBL-1	Wastewater	60%	
<i>Cladophora fascicularis</i>	Wastewater	95%	40

which is important in maintaining the microbial viability and growth¹⁹. MgSO_4 has also influenced the biosorption process with maximum biosorption of 86.1% at 0.025% MgSO_4 at biomass dosage of 3.1 mg/mL dry cell mass (Figure 3c). Mg acts as cofactors of various vital enzymes controlling cellular metabolism and growth²⁰. 0.1% Na_2MoO_4 produced maximum biosorption of 88.2% at biomass dosage of 3.8 mg/mL dry cell mass (Figure 3d). The effects of Co is very well known, it is a cofactor of vitamin B12 and plays vital role in cell growth but at higher levels it is inhibitory²¹. In our study we got maximum biosorption of 88.2% at CoCl_2 concentration of 0.2% at biomass dosage 3.6 mg/mL (Figure 3e). Copper is also an essential micronutrient but at higher dosages it shows growth inhibitory effects^{22, 23, 24}. In our study we got that optimum biosorption of 88.8% occurs at 0.2% CuCl_2 at biomass dosage of 3.8mg/mL (Figure 3f). Thus at the end of our optimization study for our strain we got maximum Cd(II) biosorption of 88.8% under optimized conditions.

There are an ample amount of studies regarding Cd(II) biosorption from aqueous medium using various biological adsorbents. The following table (Table 1) shows the latest trends in Cd(II) biosorption process.

Adsorption Isotherm and Kinetics: Langmuir and Freundlich adsorption isotherms are most commonly used models. The calculated Langmuir and Freundlich isotherm constants by linear plotting (Figure 2a,b) are shown in Table 2. From the plot (Figure 4a, b) we can see that the R^2 value for Langmuir model is greater than that

obtained by the Freundlich one. The R_L values also lies between 0 and 1 (Table 2). From the above observation we can conclude that Cd(II) biosorption by the strain is well fitted to the Langmuir model, that is monolayer adsorption is taking place under the optimized conditions.

Table 2: Langmuir and Freundlich Isotherm Constants for Cd (II) Adsorption on *Candida tropicalis* XTA 1874

Langmuir	Under Optimized Conditions
$q_{\max}$ (mg/g)	304.878 (Q1)
K_L (L/mg)	0.026171
R_L	0.071-0.72
R^2	0.9872
Freundlich	
K_F (mg/g)	9.167692
n	1.262865
R^2	0.9706

Adsorption kinetics defines the rate at which Cd(II) is adsorbed on the surface of the strain. Kinetic studies of Cd(II) adsorption has been carried out using 500mg/L of Cd(II). Adsorption kinetics has two phases, a rapid removal phase and a slower phase before reaching equilibrium. Rapid adsorption occurs at the first 80 minutes after which the rate decreased before reaching the equilibrium.

The linear plots of the kinetic models have been represented in Figure 5a,b. The calculated q_e values derived from the pseudo first order kinetic model differed significantly from that obtained by experiment. The calculated q_e values are very close to the experimental q_e values in the pseudo second order model (Table 3). Henceforth, it can be concluded that Cd(II) biosorption data best fitted to the pseudo second order model for *Candida tropicalis* XTA 1874. This suggests that the mechanism of the biosorption process depended both on the Cd(II) concentration and the type of the biosorbent

along with the rate limiting step occurred due to chemisorption^{41,42}. The value of the pseudo second order rate constant (k_2) was obtained from the plot t/q_t vs t .

Conclusion: From the present study we can ascertain that the physico-chemical factors significantly affect the biosorption process by the strain. After studying the interference from some heavy metals such as lead, copper and nickel, it has been found that the metals have no interference in Cd(II) biosorption capacity by the strain. Maximal adsorption is favored at slightly acidic pH that favors yeast growth and biosorption. Higher alkaline environment aggravates removal by hydroxide precipitation. Among the micronutrients such as CuCl_2 showed maximum impact on the biosorption process. At last we got 88.8% Cd(II) biosorption with 304.978 mg/g maximum cadmium(II) biosorption under optimized conditions. Among the carbon and nitrogen sources, glucose and urea

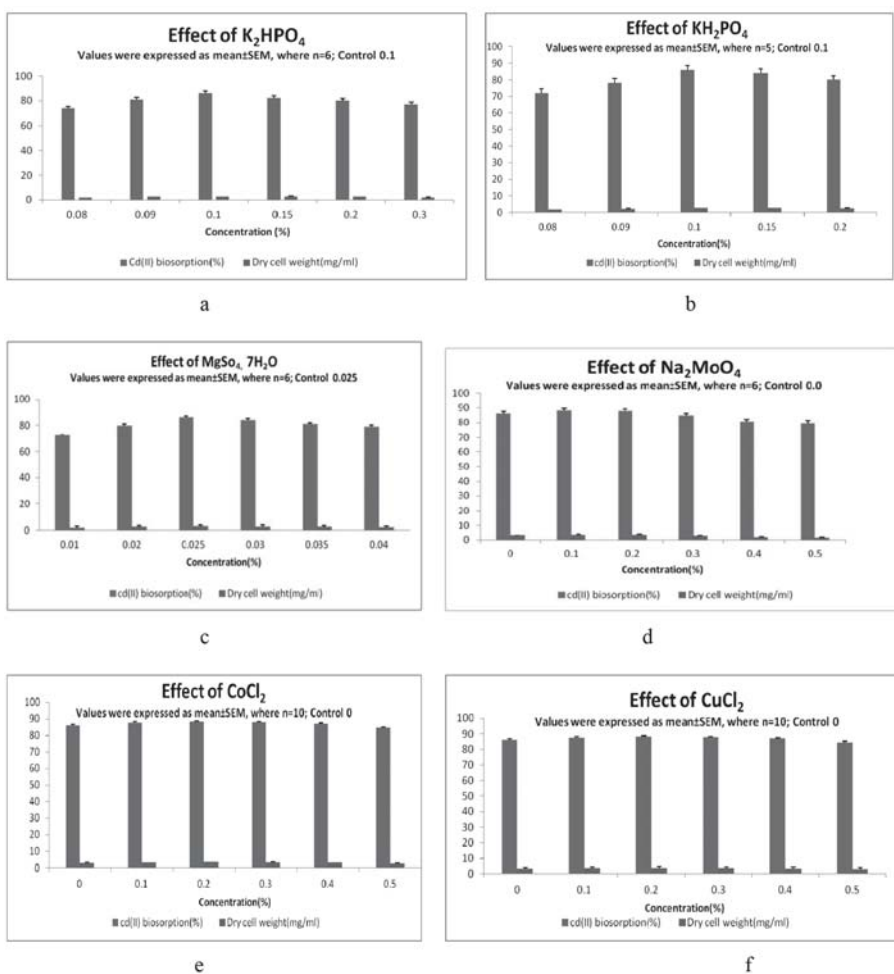
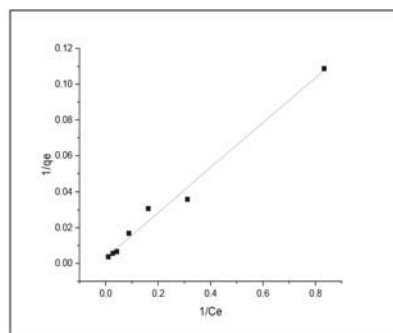


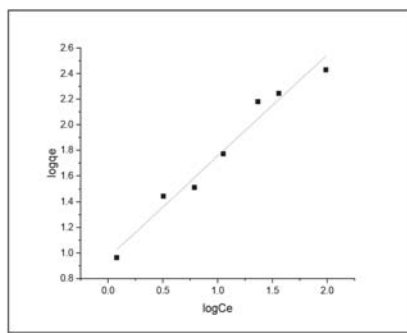
Figure 3: Effects of micronutrients on Cd(II) biosorption by *Candida tropicalis* XTA1874. (a) K_2HPO_4 (b) KH_2PO_4 (c) $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (d) Na_2MoO_4 (e) CoCl_2 (f) CuCl_2

Table 3: Values of the Parameters of Kinetic Models for Cd(II) Adsorption on *Candida tropicalis* XTA 1874

Metal ion (ppm)	Pseudo-first-order			Pseudo-second-order			
	q_e , exp (mg/g)	k_1	R^2	q_e , cal (mg/g)	k_2	R^2	q_e , cal (mg/g)
500	268.51	-0.000043	0.304	3.03	0.022911	1	268.0965

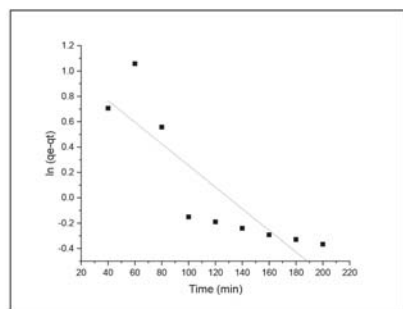


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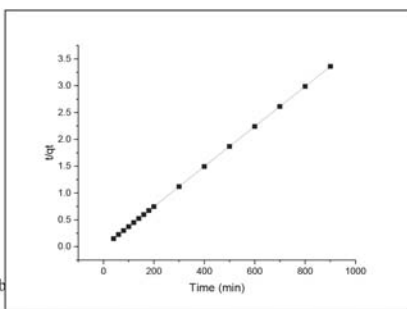


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Figure 4: Langmuir and Freundlich isotherm plots for biosorption of Cd(II) on *Candida tropicalis* XTA 1874 under optimized conditions



a



b

Figure 5: Pseudo-First and Second Order kinetic plots for Cd(II) adsorption on *Candida tropicalis* XTA 1874

showed maximal biosorptive removal of Cd(II). Thus it can be concluded that most effective Cd(II) biosorption will be achieved by maintaining all the optimum conditions and can be used effectively for removal of Cd(II) from contaminated wastewater at large scale.

Authors' Contribution: The experiment was conceptualized and designed by Dr. Subhadeep Ganguly. The experiment was conducted by Kaustav Bhattacharyya.

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