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Remediation of cefdinir from aqueous solution using pretreated dead yeast *Candida* sp. SMN04 as potential adsorbent: An equilibrium, kinetics and thermodynamic studies

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ABSTRACT

Cefdinir, a semi-synthetic third generation cephalosporin antibiotic being considered as an emerging pollutant which demands removal from environment. This work represents the biosorptive removal of cefdinir from aqueous solution using dead biomass of Candida sp. SMN04 as an adsorbent. Batch adsorption studies were conductedto determine the effect of various parameters viz. pH, biosorbent dosage, contact time and initial cefdinir concentration during cefdinir adsorption. The adsorbent was subjected to various pretreatments. The pretreatment with succinic acid showed maximum cefdinir removal of 81.0% compared to other pre-treatments. Among the various isotherms tested, Langmuir model fitted best showing maximum adsorption capacity 238.09 mg/grepresenting monolayer and homogeneous mode of adsorption, which was confirmed by SEM analysis. The kinetic data were found to follow pseudo-first order model suggesting physisorption as an underlying mechanism. Thermodynamic studies showed that the adsorption process was exothermic and spontaneous in nature. FT-IR analysis showed the involvement of functional groups viz., hydroxyl, carboxyl, amine and sulfide groups during cefdinir adsorption. Based on the results, it may be concluded that, the dead biomass of Candida sp. SMN04 can serve as potential adsorbent for the removal of cephalosporin antibiotics from aqueous environment.

Keywords: Biosorption, *Candida* sp. SMN04, Cefdinir, Isotherms, Thermodynamics.

INTRODUCTION

Antibiotics are the most successful family of drugs so far developed for improving health aspect in preventing and treating human and animal associated infections. The world's largest antibiotic production is around 5 x 10^7 kg per year, from which 3x10^7 kg are represented by the group of β-lactams [1]. Due to its wide production and application, antibiotic compounds are found to be released in large amounts as active pharmaceutical ingredients (API) in natural ecosystems [2].Average antibiotic concentrations in the receiving water bodies vary between nanograms to micrograms per litre level, while their concentrations can be up to milligrams to kilogram level in the treatment sludges because ofthe accumulative behaviour of these persistent compounds [3, 4].In general, waters containing antibiotics is becoming as an emerging issue in recent years [5].

Cefdinir is an advanced-generation, broad-spectrum cephalosporin antibiotic that has been approved for the treatment of community-acquired pneumonia, acute chronic bronchitis, acute maxillary sinusitis, pharyngitis/tonsillitis, acute bacterial otitis media, mild skin and skin-structure infections in adult and paediatric patients [6].The presence of high concentration of cephalosporin in the environment leads to a very high chemical oxygen demand, which increases the toxic strength of the effluent [7].Additionally, it contributes to an increase in the ratio of generation of cephalosporin-resistant microorganisms in the environment [8]. Therefore, there is a need to develop an eco-friendly treatment process, which may help in removing antibiotic compounds from the polluted environment.

Adsorption is considered to be the most effective and efficient method for removal of pollutants having numerous advantages including applicability, suitability for batch and continuous processes, ease of operation, possibility of regeneration, reusability and low capital cost [9, 10]. There are reports on adsorption of cephalosporin antibiotics using various adsorbents like activated carbon, nanoparticles, ion-exchange resins etc., which are havingseveral drawbacks such as limited success, high cost of the adsorbent, production of toxic compounds, large sludge generation, etc [11, 12, 13].

So far, no report is available on the use of dead yeast biomass for the removal of cephalosporin antibiotics.The present study is focused on the following objectives: (i) to explorethe potentiality of dead yeast *Candida* sp. SMN04 as an adsorbent forcefdinir removal, (ii) to study the effect of process parameters on adsorption process, (iii) to analyse the effect of pre-treatments on cefdinir uptake, (iv)to study the applicability of various isotherm and kinetic models on cefdinir adsorption and (v) to elucidiate the adsorption mechanism through FT-IR and SEM analysis.

MATERIALS AND METHODS

The yeast strain used in the present study was isolated from pharmaceutical effluent and maintained in our laboratory at 4 °C with periodic sub-culturing as mentioned in our previous study [14]. Theisolate was identified at the molecular level by 18S rDNA, ITS regions and D1/D2 domains and named as, *Candida* sp. SMN04 using a BLAST (Basic Local alignment Search Tool) similarity search in the database available on the NCBI website. The sequences were submitted to GenBank under the accession no. KF963314.1.

Preparation of biosorbent

Yeast culture pre-grown for 48 hwas centrifuged at 8400 xg for 10 min. The cells obtained as pellet were washed twice with phosphate buffer and dried to a constant weight at 95°C for 45 min. The obtained dried yeast biomasswas ground to fine powder sieved to a particle size of 150-300µm and used as native biosorbent.

Preparation of standard cefdinir solution

Cefdinir used in the study was of commercial quality (≥95% purity, Sigma Aldrich, India; Mol. wt- 395.41; M.F- $C_{14}H_{13}N_5O_5S_2$) and used without further purification. Stock solution (10^4 mg/L) was prepared by dissolving accurately weighed quantity of cefdinir in dimethyl sulphoxide (DMSO) procured from SRL, India.

Pretreatmentof biosorbent (dried yeast biomass)

The dried native yeast biomass used as biosorbentwas treated withvariouspre-treatment chemicals *viz.*, formic acid, succinic acid, sodiumdodecylsulphate (SDS) and ethylenediaminetetra acetic acid (EDTA) at a concentration of0.1M.The pre-treated biomass wasdehydrated at 60 °C for 24 h in a hot air oven. The oven temperature was raised to the desired temperature (60–140 °C) for various reaction times (0.5–4 h). The surface modified biomass was allowed to cool at room temperature, washed for several times in distilled water until the pH of the distilled water became constant. The washed yeast biomass was finallydried in an oven at 60 °C for 24 h, preserved in a desiccator as a pre-treated biosorbent.

Batch biosorptionstudies

Batch experiments were performed to demonstrate the removal of cefdinir onto untreated native biomass.Influence of process parameters such as pH (3.0-9.0), biosorbent dosage(1-9 g/L), initial cefdinir concentration(50-350 mg/L) and contact time (30-300 min)was evaluated. Experiments were conducted in Erlenmeyer flasks (250 mL) containing 100 mL working volumewith aninitial cefdinir concentration of 50 mg/L. The pH of the solution was monitored using 0.1 N HCl or 0.1 N NaOH solutions.Biosorbent (1g)was added to the flasks and shaken in orbital shaking incubator maintained at a constant speed of 140 rpm for 12 h. Blank solutions were run under same condition. All the experiments wereperformed in triplicate. The samples were taken out at definite time intervals and centrifuged at 8400 xg for 10 min in order to analyse the residual cefdinir concentration in thesolution.The amount of cefdinir adsorbed per unit adsorbent (mg of cefdinir per g of dried yeast biomass) was calculated using the following equation:

$$Q_{eq} = \frac{C_0 - C_{eq}}{M} \times V \quad (1)$$

The cefdinir removal (%) was calculated using the following equation:

$$\text{Removal (\%)} = \frac{C_0 - C_{eq}}{C_0} \times 100 \quad (2)$$

where, Q_{eq} is the equilibrium uptake, C_0 and C_{eq} are cefdinirconcentration initially and at equilibrium respectively. M is the mass of the biosorbent(g) and V is the volume of the working solution (mL).

Equilibrium and kinetic studies

The equilibrium data were analysed using isotherm models viz. Langmuir [15], Freundlich [16], Temkin [17] and Dubinin–Radushkevich [18]. Kinetic experiments were conducted under optimized conditions and samples were withdrawn at regular intervals for analysis. Pseudo-first order [19], pseudo-second order [20], intraparticle diffusion [21]and Elovich [22] models has been used for modelling the kinetic data for adsorption of cefdinir on dead yeast biomass.

Thermodynamic studies

The Gibbs free energy, enthalpy and entropy (ΔG, ΔH, ΔS) for the adsorption process were obtained from the experiments carried out at different temperatures using the following equations:

$$\log \frac{q_e}{C_e} = \frac{\Delta S}{2.303R} - \frac{\Delta H}{2.303RT} \quad (3)$$

$$\Delta G = \Delta H - T\Delta S \quad (4)$$

where,q_e/C_e is called the adsorption affinity, which is the ratio of amount of cefdiniradsorbed per unit mass to the solute concentration in unit volume of the solution at equilibrium. The values of ΔH and ΔS were determined from the slope and the intercept of the linear plot of log (q_e/C_e) Vs 1/T. These values were used to calculate ΔG, which is the fundamental criterion of spontaneity. Reaction occurs spontaneously at given temperature if the value of ΔG is negative.

Instrumental analysis

For FT-IR analysis, Fourier transformed infrared spectra were recorded in the scanning range of 500-4000 cm^{-1} on an IR-affinity 1, Shimadzu FT-IR spectrophotometer. 5mg of biosorbent before and after pre-treatmentwas encapsulated in 400 mg of KBr translucent discs, which were obtained by pressing the ground material with the aid of a bench press. Each experiment was repeated at least twice, both producing good results.

The surface morphology of the biosorbent before and after cefdinir adsorption was analyzed using scanning electron microscopy (SEM) (Stereo Scan LEO, Model-400).

RESULTS AND DISCUSSION

The dried biomass of yeast *Candida* sp. SMN04 was used as biosorbent for the removal of cefdinir from aqueous solutions. Batch experiments were conducted to study the effect various parameters during cefdinir adsorption as shown in Figure 1(a-d).

Effect of pH

The most important parameter influencing the sorption capacity is pH, which influences the biosorption process by affecting the surface charge of adsorbent and degree of ionization and speciation of the adsorbate [23]. From the Figure 1(a), the biosorption efficiency of the biomass was found to be low at acidic and kept on increasing as the pH was increased further [24]. The maximum adsorption was noted at pH of 8.0 and hence it was considered as optimum. At alkaline pH, the protonation of their surface charge decreases by neutralizing the positive charge facilitating an easier diffusion of adsorbate from the aqueous solution onto the adsorbent [13].

Effect of biosorbent dosage

Biosorbent dosage is one of the parameter that affects the sorption capacity.Figure 1(b) shows the cefdiniruptakeat different biosorbentdosage in the range of 1.0-9.0 g/L. The uptake of cefdinir increased with an increase in biosorbent dosage due to the availability of more number of binding sites on the biosorbent [13]. Maximum cefdinir adsorption was found at 5 g/L. Further increase in the biosorbent dosage did not show any improvement in the biosorption capacity due to the binding of almost all the ions to the sorbent and the establishment of equilibrium between the ions bound to the biosorbent and those remaining unsorbed in the solution.

Effect of initial cefdinirconcentration

The effect of initial cefdinir concentration onto *Candida* sp. SMN04 dried yeast biomass was studied by varying the cefdinir concentration ranging from 50-300 mg/L. The amount of cefdinir uptake by yeast biomass increased with an increase in cefdinir concentration and remained constant after equilibrium time. Maximum cefdinir uptake by dried yeast biomass was noted at 300 mg/Las shown in Figure 1(c). The sorption process was rapid at the earlier stages

and gradually decreased with the adsorption process.Liu et al. [11] reported theadsorption of cephalexin at a maximum concentration of 32 mg/L using impregnated activated carbon. The biosorptive removal (73.2 %) of pharmaceutical compounds at initial concentration of 20-500 µg/L using mixed microbial culture as sorbent was also reported [24].

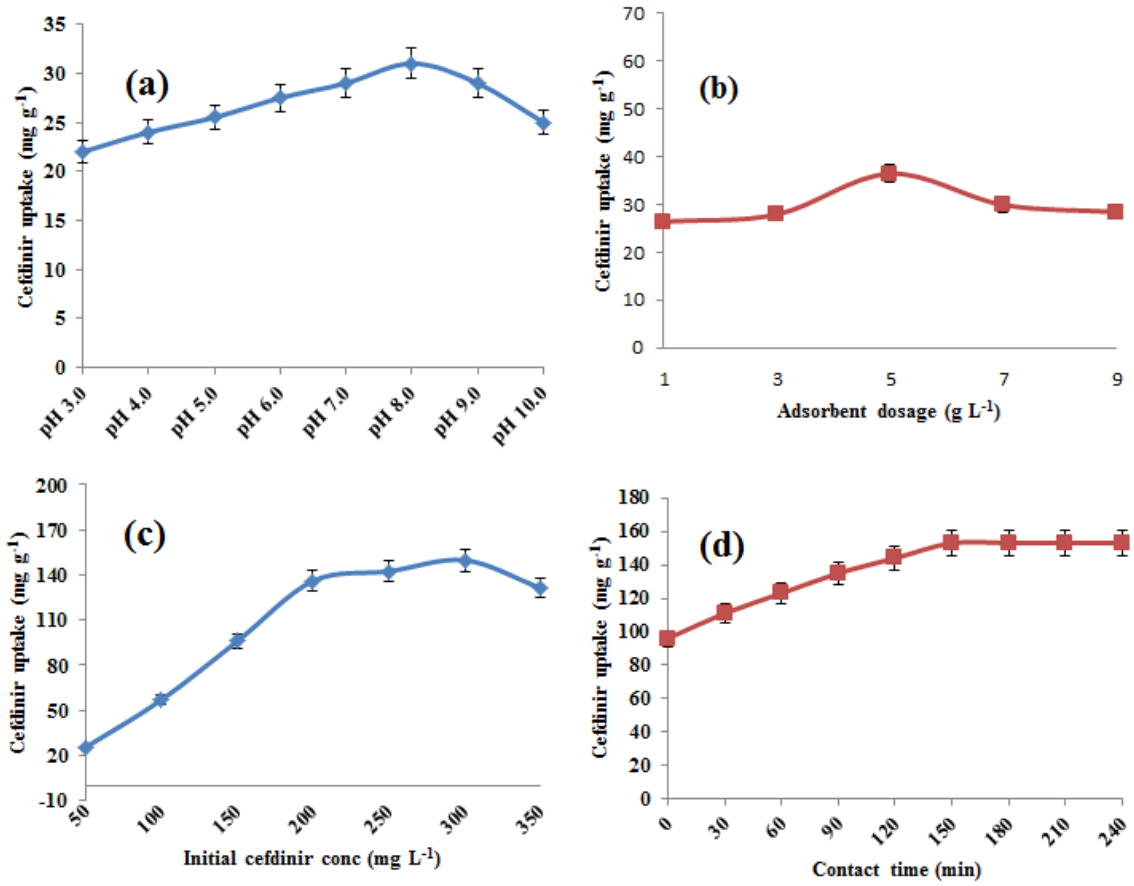

Figure 1: Effect of process parameters on biosorption of cefdinir onto dried *Candida* sp. SMN04. (a) Effect of ph, (b) Effect of biosorbent dosage, (c) Effect of initial concentration and (d) Effect of contact time. Error bars on the curve represent standard deviation of triplicate samples ($p \leq 0.0001$)

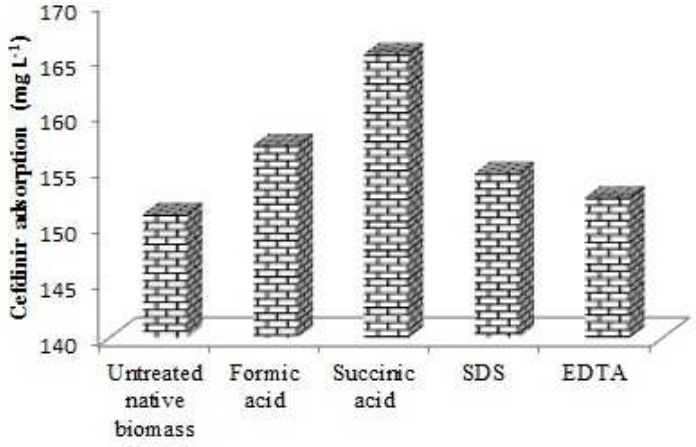

Figure 2: Comparison of cefdinir adsorption capacities for various pre-treated *Candida* sp. SMN04

Effect of contact time

The contact time is one of the important parameter for rapid sorption process. The uptake of cefdinir by dried yeast biomass increased with the increase in contact time. The amount of cefdinir uptake by the adsorbent was rapid until 180 min and thereafter, the process proceeded at slower rate and attained saturation from 210 minas shown in Figure 1(d). The rate of removal of the adsorbate is higher in the beginning due to the large surface area availabilityand few active sites on the surface of the adsorbent [13].As the time increased, the binding sites available on the biomass

surfaces are filled up and thus thecefdinir uptake capacity was decreased. The decline may be due to the decrease in the total biosorbent surface area and less available sites.

Effect of pre-treatment on cefdinir uptake

The organic acid pre-treatment may introduce the additional functional groups in biomass which may enhance the sorption capacities of the biomass [25, 26]. Figure 2 shows the effect of various pre-treatment chemicals on cefdinirbiosorption by yeast biomass of *Candida* sp. SMN04. The increase in the cefdinir uptake after pre-treatment was noted in the order: succinic acid > formic acid > SDS > EDTA> untreated native biomass. The maximum improvement in cefdinir uptake was noted as 165.38 mg/gin case of succinic acid pre-treated biomass. Thus, succinic acid treated dried yeast biomass was used for further studies.

Equillbriumstudies

Equilibrium studies were conducted to get a deeper insight on the equilibrium obtained between the amount of cefdinirbiosorbed and the residual cefdinir content. Among the various isotherm models, Langmuir isothermwas found to be the best fitted one for untreated as well as succinic acid pre-treated dead yeast biomass(Figure 3a) owing to their high R^2 values as 0.990 and 0.991 respectively as shown in Table 1, which suggested homogenous monolayer mode of adsorption. Theother isotherm models viz.Freundlich, Temkinand Dubinin–Radushkevichexhibited a poor fit owing to their low R^2 values.

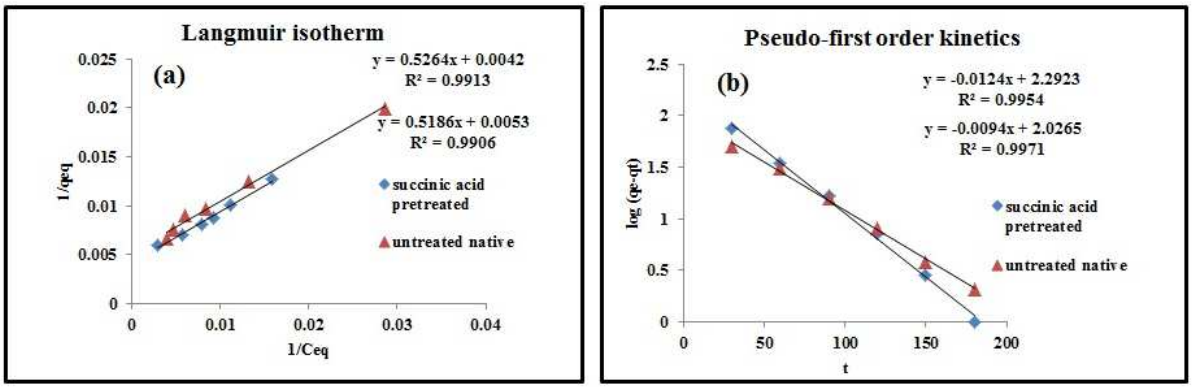

Figure 3: (a) Langmuir isotherm model and (b) Pseudo-first order kinetic model for cefdinir removal using untreated native and succinic acid pre-treated dried *Candida* sp. SMN04

Kinetics studies

Information on the kinetics of pollutant uptake is required for selecting optimum operating conditions for full-scale batch process. The kinetic constants for pseudo-first order, pseudo-second order, intra-particle diffusionandElovich modelsare presented in Table 1. Results suggested that pseudo-first order model exhibited the best fit among all the models owing to the high correlation coefficient values (0.9971 and 0.9954) for untreated and succinic acid treated biosorbent. The suitability of pseudo first order model suggested the involvement of physical mode of adsorption (Figure3b). Other kinetic models showed a poor fit due to theirlow R^2 values.

Thermodynamic studies

The biosorption process was found to be spontaneous for both untreated native and succinic acid pre-treated biosorbent as indicated by negative ΔG values with a maximum spontaneity was noted at 20 °C. The values of ΔH and ΔS were calculated from the slope and intercept of the plot of log (q_e/C_e) Vs1/T. The results indicated that the process was exothermic or heat liberating in nature as indicated by thenegative values of ΔH (-138.91 kJ/mol) for native and pre-treated (-60.45 kJ/mol) yeast biomass. Decrease in randomness at the solid/solution interface was suggested by the negative value of ΔS.

Instrumental analysis

FT-IR spectra of the native (untreated) and pre-treated dried *Candida* sp. SMN04 biomass before and after cefdinirbiosorptionare shown in Figure 4a and Figure 4b**.** The untreated native spectra showed sharp peaks at 3253.91 cm^{-1} and 3194.12 corresponding to hydrogen bonded –OH stretch of alcohol and carboxylic acid. Additionally, sharp peak at 2933.01, 1631.78 and 1022.27 cm^{-1} contributing to $–CH_2$ vibrations, N-H bond for primary amines and C=O stretch of alcohols, acids were also noted. A sharp peak at 518.85 cm^{-1} attributing to S-S disulphide stretch in amino acids was also noted. Similarly, in case of pre-treated spectra, the most significant peaks of the adsorbent were observed in the regions with a shift from a sharp peak at 3253.91 and 3194.12 to a broad peak at 3271.27 cm^{-1} range which can beattributed to hydrogen bonded –OH stretch attributing to alcohol and carboxylic

acid groups . The other peaks such as 2926.01 cm^{-1}, 1633.71 cm^{-1} and 1217.08 cm^{-1} range contribute to $–CH_2$ vibrations,N-H bond for primary amines and C=O stretch of alcohols and carboxylic acids. Another peak shift from 518.85 to 555.50 cm^{-1}stretch were found to contribute to S-S disulphide bonds in amino acids.From the results, it can be concluded that, the succinic acid pre-treatment induced no significant changes in the characteristic absorbance bands but significant peak shifts were noted [25]. Thus, the FT-IR results signified the presence ofhydroxyl, carboxyl, amine and sulfide groups on the native biosorbent surface and they were found to play an important role for cefdinir adsorption onto the biomass surface.

Table 1: Equillibrium isotherm model and Kinetic model parameters of cefdinir adsorption on native and pre-treated dried biomass of *Candida*sp. SMN04

	Parameters	*Candida* sp. SMN04	
		Untreated native	Succinic acid pre-treated
Isotherm models			
Langmuir	Q_{max} (mg/g)	188.67	238.09
	K_L (L/g)	9.65 x10^{-3}	7.604 x10^{-3}
	R^2	0.990	0.991
Freundlich	K_F (mg/g)	119.91	27.675
	n	1.87	3.27
	R^2	0.989	0.945
Temkin	A_T (L/mg)	0.028	0.293
	b_r	62.20	70.62
	B (J/mol)	39.164	34.49
	R^2	0.897	0.880
Dubinin-Radushkevich	Q_{max} (mg/g)	199.25	130.67
	K_D	1X 10^{-4}	7X 10^{-5}
	E (KJ/mol)	0.707	2.87
	R^2	0.6042	0.6471
Kinetic models			
Pseudo-first order	Q_e (mg/g)	106.29	196.01
	K_1 (per min)	0.021	0.028
	R^2	0.9971	0.9954
Pseudo-second order	Q_e (mg/g)	270.02	222.21
	K_1 (g/mg/min)	5.25x10^{-4}	6.49x10^{-5}
	R^2	0.9682	0.9684
Intra particle diffusion	K_{int} (mg/g)	7.525	0.1032
	C	50.753	4.077
	R^2	0.9783	0.9882
Elovich	α (mg/g/min)	13.742	18.498
	β (g/mg)	0.0358	0.0491
	R^2	0.9284	0.9326

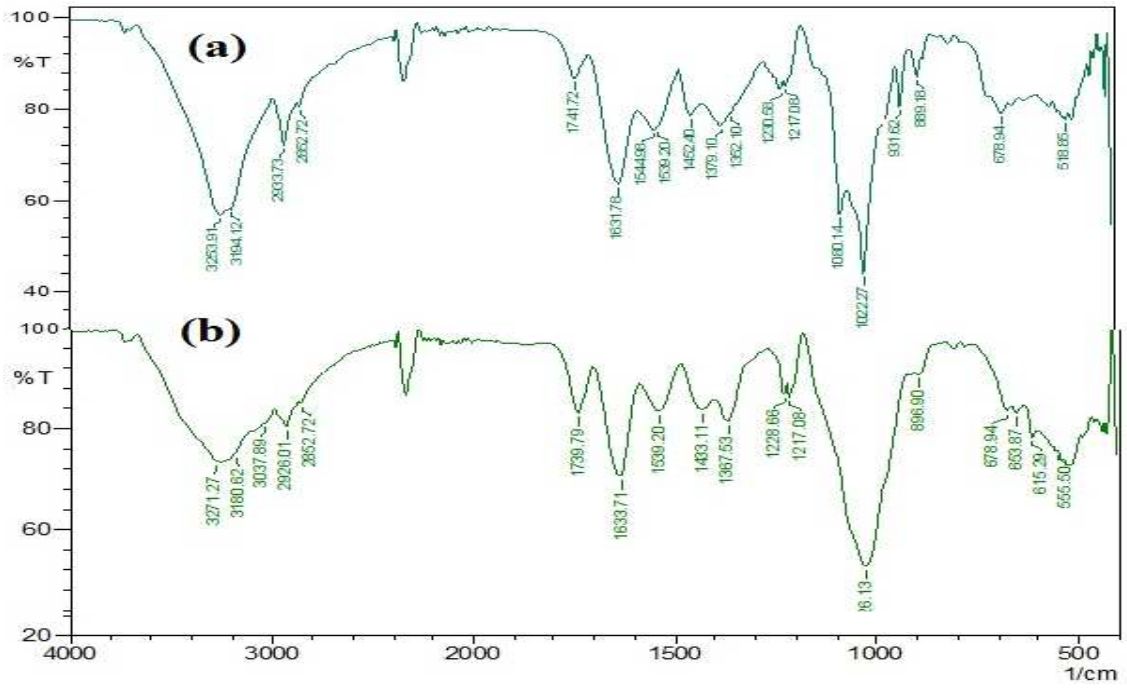

Figure 4: FT-IR spectra of untreated native and succinic acid pre-treated dried

***Candida* sp. SMN04 after cefdinir adsorption.**

SEM analysis revealed the surface topology of native (Figure 5a) and succinic acid pre-treated (Figure 5b) dried yeast biomassafter cefdinirbiosorption. As shown in Figure 5(a), native biosorbent showed a smooth appearance confirming homogeneous mode of cefdinir adsorption. Pre-treated biosorbentshowed porous and irregular surface structure confirming more amount of cefdinir adsorption (Figure 5b).

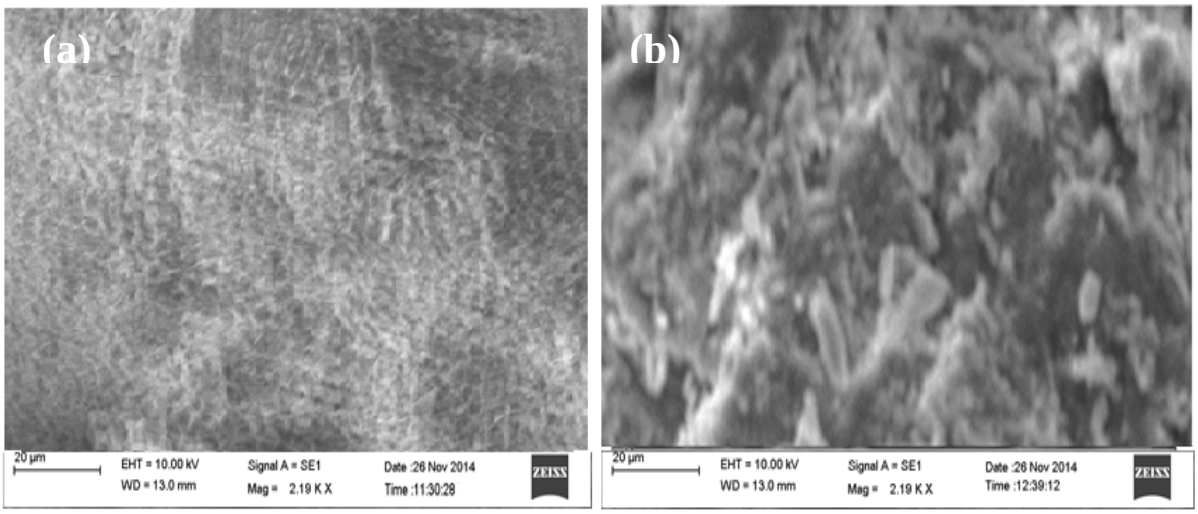

Figure 5: SEM analysis (a) untreated native and (b) succinic acid pre-treated dried *Candida* sp. SMN04 after cefdinirbiosorption

Based on the reported works as shown in Table 2, it can be seen that succinic acid treated dead yeast biomass can serve as eco-friendly and potential adsorbent for the remediation of cephalosporinantibiotics from aqueous environments.

Table 2: Reported works on adsorption of pharmaceutical compounds including cephalosporin antibiotics

Pharmaceutical Compounds	Adsorbents	Initial conc of antibiotics (mg/L)	Removal (%)	References
Cefradine, Cefalexin, Ceftazidime, Cefixime	Alga-activated sludge	100	89.9 94.9 89.7 100.0	[27]
Sulfamethoxazole, Carbazepine, Caffeine,	Mixed microbial culture	100	73.2 4.2 5.3	[24]
Cephalexin	Original and metal ion modified Activated carbon	4-32	100	[11]
Cephalexin, Cefixime	MgO nanoparticles	300	31.3	[13]
Cefdinir	**Dried succinic acid treated yeast biomass**	**300**	**81.0**	**Present study**

CONCLUSION

The present study is the first report on the application of dried yeast biomass *Candida* sp. SMN04 as biosorbent for the removal of cefdinirfrom aqueous environment. Untreated native biosorbent showed cefdiniradosorption151.02 mg/gunder optimized condition which was found to be enhanced up to 238.09 mg/gafter succinic acid pretreatment.Maximum cefdinir removal (81.0%) was noted in case of succinic acid pre-treated yeast biomass. Equilibrium studies suggested a homogeneous monolayer mode of adsorption to be the underlying phenomena. Kinetic and thermodynamic studies defined the physical and exothermic mode of adsorption process. It may be concluded that, the dried yeast biomass can serve as potential agent for the removal of cephalosporin antibiotics from pharmaceutical wastewater.

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