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Potential of *Saccharomyces cerevisiae* flocculent strain to biosorb copper and cadmium ions from aqueous solution

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ABSTRACT

Copper and cadmium are highly toxic metals that pose deleterious effects on the biota upon entering the water bodies. Therefore, there is a dire need to remove both these metal ions from water bodies. Considering this, the present study was planned to evaluate the biosorption behavior of *Saccharomyces cerevisiae* for removal of copper and cadmium from aqueous solution. Maximum sorption of both metal ions was observed at temperature (25ºC), dose (8 ml/100 ml equivalent to 0.2 g/100 ml) and contact time (48 h). Equilibrium data of biosorption study was suitably described by the Langmuir isotherm model with maximum biosorption capacity of 15.87 mg/g and 13.33 mg/g for copper and cadmium, respectively. The results of thermodynamic studies confirmed endothermic as well as spontaneous nature of biosorption process which also followed pseudo second-order kinetics for both copper and cadmium ions. Considerable variations in physico-chemical characteristics of biosorbent materials were observed using SEM, SEM-EDX, CHNS analyzer and XRD before and after biosorption process.

Keywords: Langmuir; Biosorbent; Biosorption; Heavy metals; Water pollution

1. Introduction

Increase in environmental contamination during recent years is one of major consequences of industrial development. Excessive release of heavy metals into water bodies due to agricultural practices, industrialization and urbanization has posed a great environmental pollution in developed as well as developing countries. Heavy metals include the metals which have specific gravity more than 5 g/cm^3 and considered as a hazardous pollutant because of their non-biodegradable properties and toxicities even at low concentrations. Moreover, these metal ions pose serious threat to human, animal and plants. Both copper and cadmium make the way to water bodies through the effluent discharge from different industries *viz.*, electroplating, electrical, storage batteries, leather tanning, pesticides, batteries and metal mining [1,2]. Higher concentration of copper causes various disorders such as gastrointestinal irritation, cirrhosis, central nervous system, hepatic and renal damage while, cadmium, a neuro toxic heavy metal can cause diseases like cancer, lung fibrosis, dyspnea, hypertension and "itai-itai". Presence of both the metal ions in the environment directly indicates their magnitude of toxicities. Due to toxic effects and bioaccumulation tendency of these metals, their presence in the environment has become a potential threat to plants, animals and human beings. Therefore, it is mandatory to remove these heavy metals from industrial effluents by adopting different treatment techniques before discharging industrial sewage into the aquatic systems. Various purification techniques available for the removal of heavy metals from wastewater include evaporation, liquid-liquid extraction, ion-exchange, precipitation, electrodialysis, phytoremediation

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and osmosis. Although these techniques are enough efficient to remove metal ions from aqueous solution but they have some limitations like of these technologies involve high cost setup, heavy instrumentation and high energy requirement. On other hand, biosorption is the one of the effective techniques used for the removal of heavy metals from wastewater which is becoming a favorite approach and has attracted widespread interest because of its high efficiency and low cost. In the past few decades, biosorption technique utilizing different micro-organisms such as yeast [3–5], fungi [6], algae [7] and bacteria [8] was used for the removal of heavy metals from wastewater. Metal uptake capacities of micro-organisms depend upon the chemistry of metal ions, cell physiology, surface properties of organism and physico-chemical influence of environment like pH, temperature and metal concentration [9]. Microorganisms such as fungi, algae and bacteria are present in soils, sediments, natural water as well as wastewater and possess capacity to interact with metal ions. Interaction between microbes and metal ions is a pre-requisite for designing effective and efficient remediation strategies [10,11].

Among the different micro-organisms, *Saccharomyces cerevisiae* is a safe micro-organism and readily available source of biomass for the removal of heavy metals from wastewater [12–14]. *Saccharomyces cerevisiae* in different forms such as flocculent and non-flocculent strain [15], living and dead cells [16], immobilized and free cells [17], wild and mutant types [18] have been used as sorbents. It is well established that yeast cells of *Saccharomyces cerevisiae* have ability to interact with heavy metals and form flocculates [19]. Therefore, the present work was planned with an objective to use yeast cells of *Saccharomyces cerevisiae* (flocculant strain) for removal of copper and cadmium from aqueous solution. Different experimental parameters of biosorption *viz.,* biosorption time, dosage and initial concentration of copper and cadmium were studied. The equilibrium, kinetic, thermodynamic models were applied to study the biosorption behavior of *Saccharomyces cerevisiae* for the removal of copper and cadmium. Physical characteristics of *Saccharomyces cerevisiae* before and after biosorption was analyzed using Scanning Electron Microscope – Energy dispersive X-ray spectroscopy (SEM-EDX), CHNO/S analyzer and X-ray diffraction (XRD).

2.Materials and methods

2.1. Saccharomyces cerevisiae

The flocculent strain of *Saccharomyces cerevisiae* (MTCC-250) was procured from MTCC institute, Chandigarh, Punjab (India) in freeze dried form. The strain was routinely maintained on culture plate containing growth medium of malt extract (3.0 mg/l), yeast extract (3.0 mg/l), peptone (5.0 mg/l), glucose (10.0 mg/l), agar (20.0 mg/l) in 1000 ml distilled water at 4°C.

2.2. Nutrient medium

Pre-culture was prepared in 100 ml of nutrient medium containing malt extract (0.3 mg), yeast extract (0.3 mg), peptone (0.5 mg) and glucose (1.0 mg) in 100 ml distilled water by inoculating nutrient medium with inoculums from culture plate in incubator shaker at 120 rpm for 48 h.

2.3. Copper and cadmium stock solutions

All the chemicals used were of analytical grade and purchased from Sigma-Aldrich. A stock solution of copper was prepared by dissolving 3.802 g of cupric nitrate ($Cu(NO_3)_2{\cdot}3H_2O$) in 1000 ml of double distilled water and stock solution of cadmium was prepared by dissolving 2.744 g of cadmium nitrate ($Cd(NO_3)_2{\cdot}4H_2O$) in 1000 ml of double distilled water.

2.4. Toxicity studies

Preliminary studies were carried out to explore the toxicity of copper and cadmium metal ions. For which, a loopful of culture was inoculated in different flasks containing 10 ml of nutrient broth inoculation with concentrations (1, 10, 25, 50, 100, 150 and 200 mg/l) of both metals individually. The solutions were kept at 25ºC in incubator shaker for 48 h. After 48 h, optical density (OD) of different solutions (against solution of nutrient broth without culture inoculation) was noted at 640 nm using spectrophotometer (Model: BioTek; Make: Synergy HT). The OD was observed to be less than 0.1 for the solution containing copper and cadmium beyond 50 mg/l, indicating their cytotoxicity. Hence, concentrations (1, 10, 25 and 50 mg/l) of both metal ions were selected for the biosorption studies.

2.5. Biosorption studies

The batch experiments were conducted to achieve a set of optimum conditions for maximum biosorption of copper and cadmium ions using *Saccharomyces cerevisiae*. Biosorption experiments were conducted systematically at different conditions using various concentrations (1, 10, 25 and 50 mg/l of copper and cadmium in 100 ml of nutrient medium contained in flasks); doses (1, 2, 4, 6, 8 and 10 ml/100 ml equivalent to 0.025, 0.05, 0.1, 0.15, 0.2, 0.25 g/100 ml); temperatures (15, 25, 35 and 45°C); contact times (6, 12, 24, 36, 48, 60 and 72 h) and pH (7) at 120 rpm on incubator shaker . After biosorption process, flasks were removed from the shaker and autoclaved at 121°C for 30 min. Then, the contents of flasks were filtered through *Whatman* filter paper No. 1 and the quantitative analysis of copper and cadmium was carried out using atomic absorption spectrophotometer (Model: AA240 FS; Make: Agilent). Percentage removal of the copper and cadmium was calculated using equations as given here under [Eq.(1)]:

$$\text{Percent removal (\%)} = \frac{C_i - C_f}{C_i} \times 100 \quad (1)$$

Biosorption capacity of biosorbent at equilibrium q_e (mg/g) was calculated by

$$q_e = \frac{(C_i - C_f)V}{W} \quad (2)$$

where C_i = initial concentrations (mg/l) of the copper or cadmium; C_f = final concentrations (mg/l) of the copper or cadmium; V = volume of the solution; W = mass of biosorbent.

2.6. Sorption isotherm models

The biosorption equilibrium data of copper and cadmium were analyzed using Langmuir [Eq. (3)], Freundlich [Eq. (4)] and Tempkin isotherms [Eq. (5)]. The linear form of Langmuir, Freundlich and Tempkin equations are given below:

Langmuir Isotherm $$\frac{C_e}{q_e}=\frac{1}{q_o b}+\frac{C_e}{q_o} \quad (3)$$

Freundlich Isotherm $$\log q_e=\log K_f+\frac{1}{n}\log C_e \quad (4)$$

Tempkin Isotherm $$q_e=B\log K_t+B\log C_e \quad (5)$$

$$B=\frac{RT}{b}$$

where C_e (mg/l) = equilibrium concentration of metal ions; q_e (mg/g) = maximum biosorption capacity of biosorbent; q_o (mg/g) = amount of metal ions biosorbed; b = Langmuir constant (rate of biosorption); K_f = Freundlich isotherm constant related to biosorption capacity; $1/n$ = Freundlich isotherm constant related to biosorption intensity; B = heat of the biosorption; R = universal gas constant; T = absolute temperature (Kelvin); $1/b$ = biosorption potential of the biosorbent; K_t (l/mg) = equilibrium binding constant corresponding to the maximum binding energy.

2.7. Kinetic models

Kinetics of biosorption of copper and cadmium onto *Saccharomyces cerevisiae* were studied by using pseudo-first-order [Eq. (6)] and pseudo-second order [Eq. (7)] sorption models.

Pseudo first order kinetic $$\log\left(q_e-q_t\right)=\log q_e-\frac{K_1}{2.303} \quad (6)$$

Pseudo second order kinetic $$\frac{t}{q_t}=\frac{1}{K_2 q_e^2}+\frac{t}{q_e} \quad (7)$$

where q_e (mg/g) = biosorption capacities of biosorbent at equilibrium; q_t (mg/g) = biosorption capacities of biosorbent at time (t); k_1 (g/mg min) = first order reaction rate constant; k_2 (g/mg min) = second order reaction rate constant; t (h) = time.

2.8. Thermodynamic studies

Thermodynamic parameters for biosorption studies:

$$\ln\frac{q_e}{C_e}=\frac{-\Delta H^\circ}{RT}+\frac{\Delta S^\circ}{R} \quad (8)$$

$$\Delta G'=\Delta H'-T\Delta S' \quad (9)$$

where q_e (mg/g) = biosorption capacitiy of biosorbent at equilibrium; C_e (mg/l) = equilibrium concentration of metal ions; ΔG° (kJ/mol)= change in free energy associated with biosorption process; ΔH° (kJ/mol) = change in enthalpy associated with biosorption process; ΔS° (J/mol K) = change in entropy associated with biosorption process.

2.9. Physical characterization

Morphological characterization of biosorbent and confirmation of biosorption of copper and cadmium on the surface of *Saccharomyces cerevisiae* was carried out using Scanning Electron Microscope (SEM). For SEM analysis, samples (before and after biosorption) were filtered with the help of *Whatman* filter paper No. 1 and biomass (residue) was dried at 60°C in hot air oven. Biomass of *Saccharomyces cerevisiae* was crushed to form fine powder using pestle and mortar. Dried powder of *Saccharomyces cerevisiae* biomass before and after biosorption was coated with gold using gold sputter unit (Model: Q150R ES, make: Quorum) for 30 min. Coated samples were analyzed for elemental compositon using SEM-EDX (EDX Model: X-Max, make: GEMINI). Carbon, nitrogen, hydrogen and sulfur content in *Saccharomyces cerevisiae* sample was analyzed by CHNS analyzer (Model: Flash 2000, make: Thermo). Crystalline phase of *Saccharomyces cerevisiae* was determined by X-ray diffraction (XRD Model: D8 FOCUS, make Bruker)using Cu κα radiation (40 kV, 40 mA) between 2θ value of 10–80°.

3. Results and discussion

3.1. Effect of pH

pH is one of the most important parameters for biosorption of metal ions. Cells of yeast cannot survive in highly acidic and highly alkaline media. Therefore, all the batch experiment sets were conducted at neutral pH 7.

3.2. Effect of dose

Percentage removal of copper and cadmium was increased with increasing dose of *Saccharomyces cerevisiae* culture from 1 ml/100 ml to 8 ml/100 ml because of the availability of more sorption sites on surface of *Saccharomyces cerevisiae*. Equilibrium was attained at dose ≥ 8 ml/100 ml. Maximum percentage removal of metal ions was found to be 63.33% for cadmium and 65.33% for copper at 8 ml/100 ml (≈0.2 g/100 ml) dose of *Saccharomyces cerevisiae*. It was observed that percentage removal of copper ions was higher than cadmium metal ions (Fig. 1a). Similar results were observed for biosorption of chromium onto baker yeast [20] and different heavy metals viz., lead, zinc, chromium, cobalt, cadmium and copper onto *Saccharomyces cerevisiae* [3].

3.3. Effect of contact time

Effect of contact time on the percentage removal of copper and cadmium was examined with different contact time ranging from 6 h to 72 h. Figs. 1b, 2 and 3 shows that percentage removal of copper and cadmium has increased with contact time upto 48 h and further increase in contact time

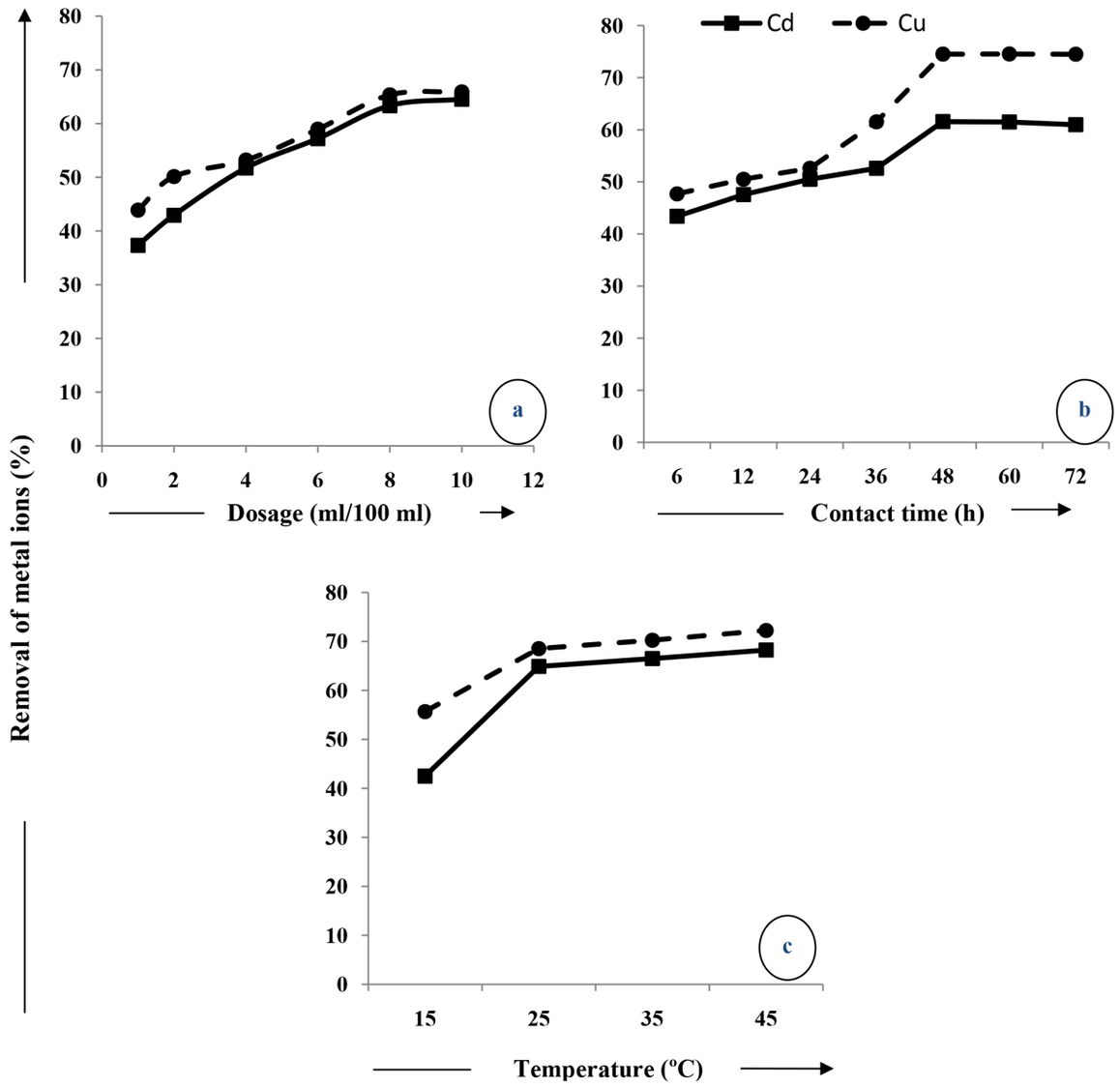


Fig. 1. Biosorption of copper and cadmium ions onto *Saccharomyces cerevisiae* with variations in (a) dosage, (b) contact time and (c) temperature.
Conditions used:
(a):- pH: 7; Contact time: 48 h; Concentration: 50 mg/l; Temperature: 25°C
(b):- pH: 7; Dosage: 8 ml/100 ml; Concentration: 50 mg/l; Temperature: 25°C
(c):- pH: 7; Contact time: 48 h; Dosage: 8 ml/100 ml; Concentration: 50 mg/l.

hardly result in more biosorption. Hence, it can be found that equilibrium was achieved after 48 h. It is well known fact that biosorbent sites are numerous at beginning of biosorption process (resulting in increased biosorption up to certain period) but after achieving the maximum biosorption, the process slows down due to non-availability of free sites on the surface of biosorbent. The time, at which the biosorption is maximum is considered to be optimum contact time. Maximum percentage removal was found to be 61.57% and 74.53% for cadmium and copper, respectively with contact time of 48 h. After 48 h, there was no further adsorption of copper and cadmium probably due to non-availability of active sites on the surface of *Saccharomyces cerevisiae* The present results revealed that percentage removal of copper ions was higher than cadmium. The results were in agreement with the work earlier done by Chen and Wang [21] and Nagy et al. [22].

3.4. Effect of temperature

Percentage removal of copper and cadmium were examined at different temperature i.e. 15°C, 25°C, 35°C and 45°C and obtained results are shown in Fig. 1c. In the present study, percentage removal of copper and cadmium was found to be increased with increasing temperature

from 15 to 45°C which probably could be due to breaking of bonds between functional groups already attached to the biosorbent. This process further provides the active sites on the surface of biosorbent to bind with the metal ions present in aqueous solution. Similar observations were made by Shu-juan et al. [24] for biosorption of cadmium from aqueous solution using waste of *Saccharomyces cerevisiae*.

3.5. Effect of initial concentration

It can be seen that percentage removal of copper and cadmium ions was decreased with increasing concentration of copper and cadmium from 1 to 50 mg/l. It was found that percentage removal of copper (Fig. 2) and cadmium (Fig. 3) ions was observed to be maximum at lowest concentration (1 mg/l). Biosorption of copper was higher than cadmium at all concentrations. Reduction in the removal of copper and cadmium was observed with increasing the concentrations of metal ions may be due to nonavailabilty of sorption sites on surface of *Saccharomyces cerevisiae* to accumulate copper and cadmium after initial biosorption process [22]. Zan et al. [23] reported that cadmium and copper ions removal efficiency decreased with increasing concentration of copper and cadmium ions using immobilized *Saccharomyces cerevisiae*.

3.6. Isotherms

Langmuir, Freundlich and Tempkin isotherms were applied to study the equilibrium biosorption process. Table 1 summarizes the different parameters of Lang-

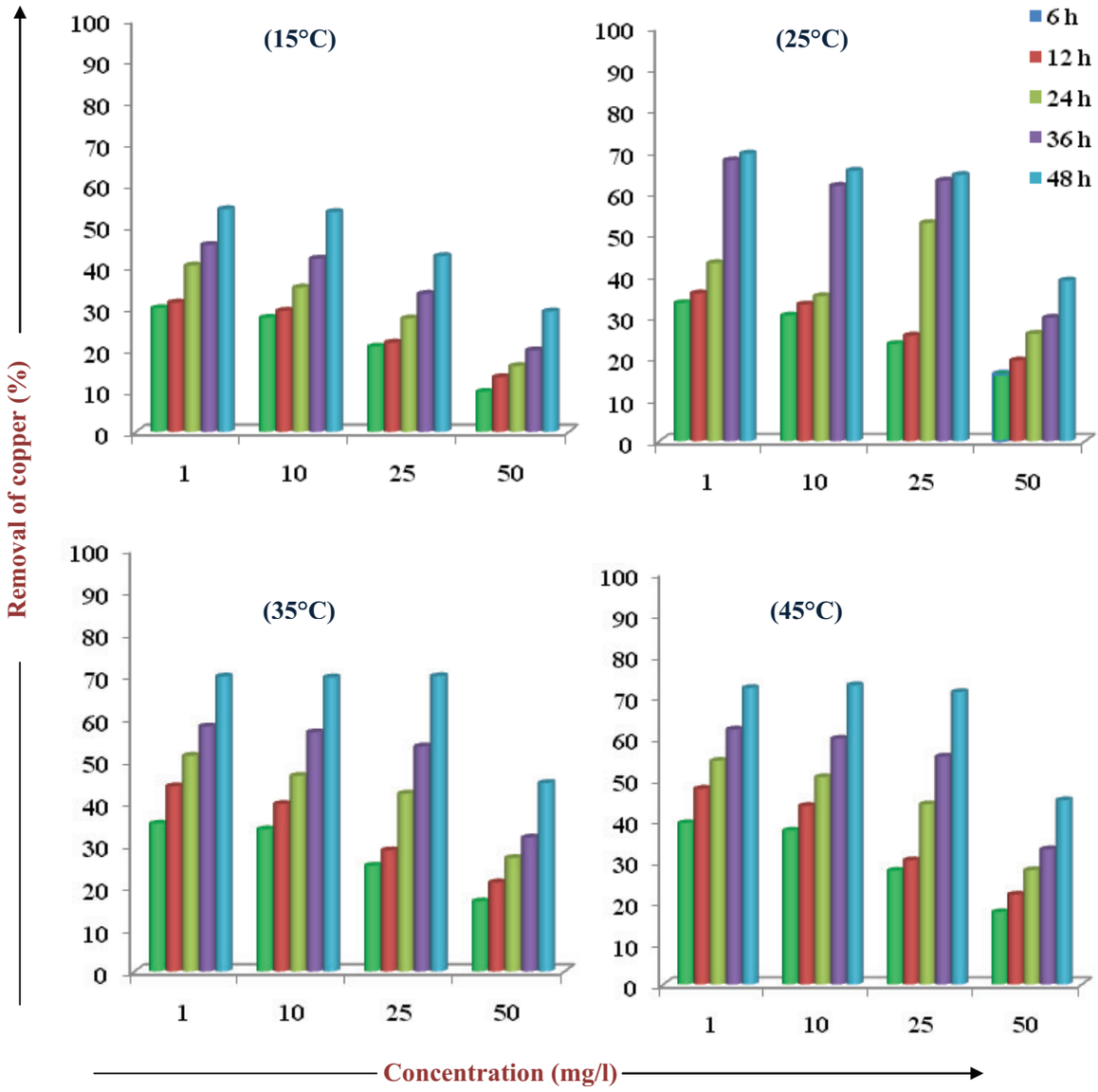


Fig. 2. Effect of different temperatures on the percentage removal of copper ions using *Saccharomyces cerevisiae*.

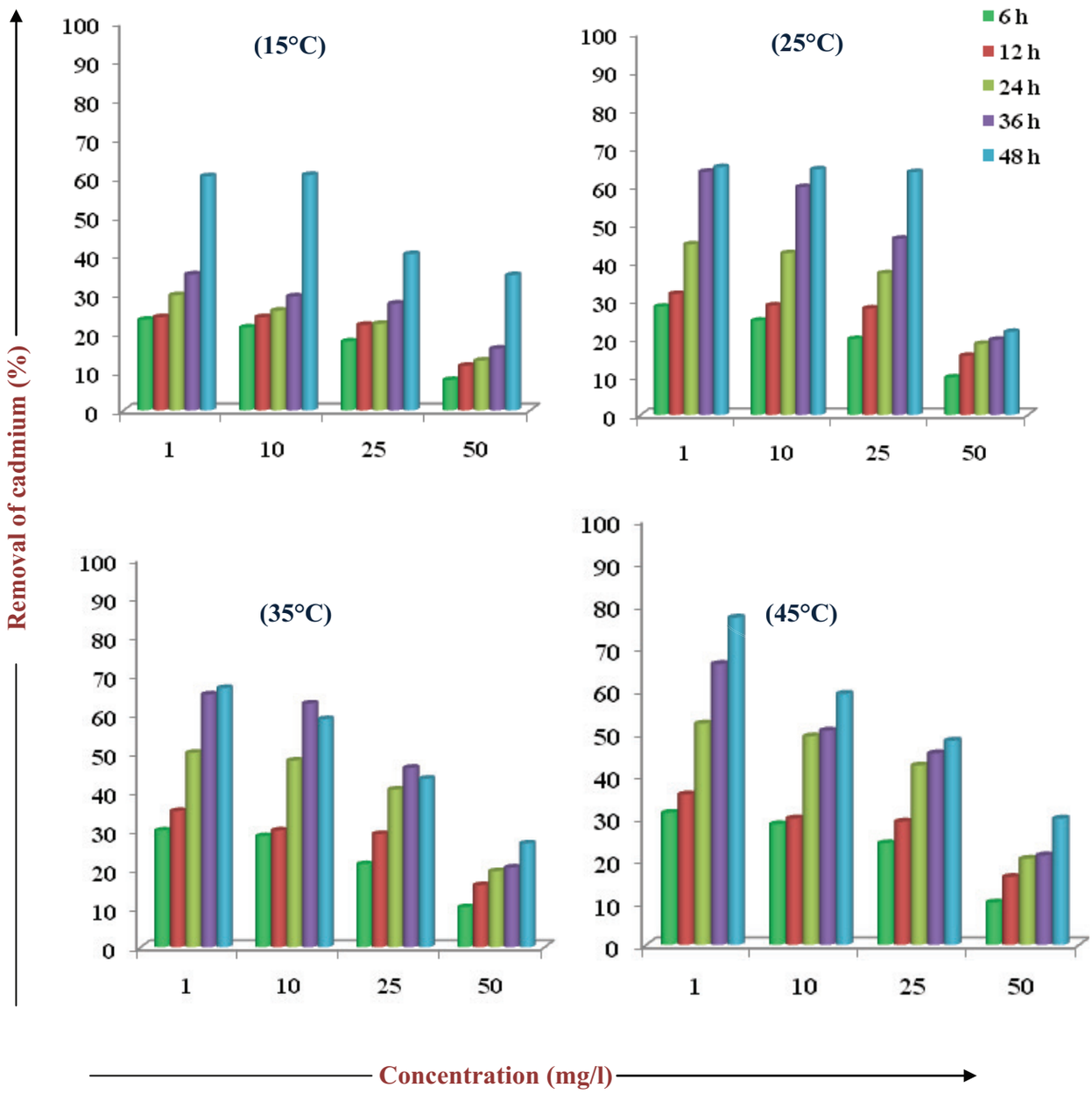


Fig. 3. Effect of different temperatures on the percentage removal of cadmium ions using *Saccharomyces cerevisiae.*

muir, Freundlich and Tempkin isotherms. It was found that maximum biosorption capacity for copper ions (15.87 mg/g) was higher than that for cadmium ions (13.33 mg/g) onto *Saccharomyces cerevisiae* indicating monolayer biosorption at 25°C temperature. At all the temperatures (15, 25, 35 and 45°C), values of R_L were less than 1 for all samples which confirmed the favorable biosorption process for copper and cadmium onto *Saccharomyces cerevisiae*.

Freundlich isotherm indicated that biosorption intensity $1/n$ value was less than 1 confirming a favorable biosorption. K_f value from Freundlich isotherm was found to be 1.180 mg/g for biosorption of copper and 1.119 mg/g for biosorption of cadmium onto *Saccharomyces cerevisiae* at 25°C temperature. Biosorption intensity ($1/n$) values obtained from copper and cadmium onto *Saccharomyces cerevisiae* less than one indicating normal biosorption process.

Tempkin isotherm showed that values of heat of biosorption were less than 40 kJ/mol indicating biosorption was physical process. Moreover, biosorption process was endothermic in nature. Higher equilibrium binding constant values at different temperature and contact time indi-

Table 1
Langmuir, Freundlich and Tempkin isotherm parameters for the biosorption of copper and cadmium onto *Saccharomyces cerevisiae*.

Isotherm models	Parameters	Temperature	Copper	Cadmium
Langmuir Isotherm	Q_{max} (mg/g)	15°C	10.86	9.433
		25°C	15.87	13.333
		35°C	14.08	9.061
		45°C	10.10	10.00
	b (l/mg)	15°C	0.061	0.128
		25°C	0.088	0.056
		35°C	0.143	0.046
		45°C	0.818	0.037
	R_L	15°C	0.246	0.135
		25°C	0.185	0.261
		35°C	0.123	0.231
		45°C	0.024	0.205
	r	15°C	0.991	0.998
		25°C	0.994	0.995
		35°C	0.994	0.999
		45°C	0.991	0.993
Freundlich Isotherm	$1/n$	15°C	0.512	0.584
		25°C	0.537	0.587
		35°C	0.786	0.506
		45°C	0.920	0.461
	K_f (mg/g)	15°C	1.227	1.143
		25°C	1.180	1.119
		35°C	1.054	1.119
		45°C	1.007	1.093
	r	15°C	0.982	0.988
		25°C	0.989	0.986
		35°C	0.980	0.981
		45°C	0.983	0.989
Tempkin Isotherm	b (l/g) (mg^{-1})	15°C	415.339	995.80
		25°C	321.762	819.17
		35°C	703.299	750.61
		45°C	474.318	654.92
	K_t (l/mg)	15°C	2.048	5.780
		25°C	2.199	3.488
		35°C	2.388	2.791
		45°C	3.789	2.738
	r	15°C	0.981	0.989
		25°C	0.982	0.983
		35°C	0.983	0.981
		45°C	0.983	0.980
	B (J/mol)	15°C	2.576	2.655
		25°C	3.554	3.126
		35°C	4.126	3.190
		45°C	4189	3.783

cated strong interactions between metal ions (copper and cadmium) and biosorbent (*Saccharomyces cerevisiae*).

Dhankhar et al. [25] reported that equilibrium data for biosorption of uranium using untreated and NaOH treated *Saccharomyces cerevisiae* was more correlated with Langmuir isotherm than to Freundlich isotherm. Similar results have been reported for biosorption of iron, lead, cadmium onto brewery waste biomass [26]; cadmium onto modified and pure strain yeast biomass [22]; lead, copper, cadmium onto brewery biomass and ethanol treated waste [27]; cadmium and lead onto baker yeast biomass [28].

3.7. Kinetic studies

Kinetic of biosorption of copper and cadmium onto *Saccharomyces cerevisiae* was studied using pseudo first and second order kinetic models and resulting parameters such as rate constants (k_1 and k_2), experimental ($q_{e(exp)}$) and calculated equilibrium biosorption capacity ($q_{e(cal)}$) and regression coefficient (r) are summarized in Table 2. Dramatic difference between experimental and calculated biosorption capacity and poor regression from first order kinetic indicated that biosorption of copper and cadmium onto *Saccharomyces cerevisiae* did not follow pseudo first order kinetics. On the other hand, a good agreement between experimental and calculated biosorption capacity value with good regression coefficient indicating that biosorption process followed pseudo second order kinetic. Similar results have been reported by other authors [23,29–30] which confirmed that biosorption of copper and cadmium using *Saccharomyces cerevisiae* was better described by pseudo second order kinetic model in comparison to pseudo first order kinetic model.

3.8. Thermodynamic studies

Thermodynamic parameters were evaluated for biosorption of copper and cadmium ions onto *Saccharomyces cerevisiae* and ΔG^o values were found to be –13.575, –14.246, –14.916, –15.586 for biosorption of copper and –11.391, –12.043, –12.695, –13.348 for biosorption of cadmium at different temperature *viz*., 288, 298, 308, 318 K, respectively (Table 3). Negative value of ΔG^o indicated biosorption process was feasible and spontaneous in nature. Positive value of ΔH^o confirmed that biosorption process was endothermic in nature for both copper (5.723 kJ/mol) and cadmium (7.396 kJ/mol) ions. The values of ΔS^o for copper (67.011 J/mol K) and cadmium (65.232 J/ mol K) were found to be positive due to the exchange of the metal in the aqueous solution which further results in more randomness during adsorption process. Positive value of ΔS^o reflects the increase in randomness during biosorption of copper and cadmium onto *Saccharomyces cerevisiae*. Positive entropy suggests high affinity of the biosorbent for the sorbate and increased randomness at the solid/liquid interface. Studies were in line with earlier reports [31–36].

3.9. Physical characterization

Fig. 4 shows the micrographs of *Saccharomyces cerevisiae* before and after biosorption of copper and cadmium ions.

Table 2
Kinetic parameters for the biosorption of copper and cadmium onto *Saccharomyces cerevisiae*

Kinetic models	Parameters	Temperature	Copper	Cadmium
Pseudo-first order	$q_{e(exp)}$ (mg/g)	15°C	7.300	8.70
		25°C	9.830	7.66
		35°C	11.16	6.66
		45°C	11.18	7.41
	$q_{e,(cal)}$ (mg/g)	15°C	5.199	4.775
		25°C	6.902	5.046
		35°C	7.533	6.501
		45°C	8.204	7.482
	K_1 (min^{-1})	15°C	0.025	0.016
		25°C	0.029	0.021
		35°C	0.029	0.018
		45°C	0.025	0.016
	r	15°C	0.932	0.947
		25°C	0.938	0.878
		35°C	0.938	0.939
		45°C	0.939	0.945
Pseudo-second order	$q_{e(exp)}$ (mg/g)	15°C	7.300	8.70
		25°C	9.830	7.66
		35°C	11.16	6.66
		45°C	11.18	7.41
	$q_{e,(cal)}$ (mg/g)	15°C	7.692	8.771
		25°C	9.804	7.692
		35°C	11.24	6.849
		45°C	11.11	7.813
	K_2 (mg/g min^{-1})	15°C	0.008	0.005
		25°C	0.009	0.007
		35°C	0.007	0.015
		45°C	0.007	0.010
	r	15°C	0.988	0.930
		25°C	0.999	0.990
		35°C	0.999	0.992
		45°C	0.999	0.964

Table 3
Thermodynamic parameters for biosorption of copper and cadmium onto *Saccharomyces cerevisiae*

Metal	Temperature	Thermodynamic parameters		
	(°C)	ΔH (kJ mol^{-1})	ΔS (J mol^{-1} K)	ΔG (kJ mol^{-1})
Copper	15	5.723	67.011	–13.575
	25			–14.246
	35			–14.916
	45			–15.586
Cadmium	15	7.396	65.232	–11.391
	25			–12.043
	35			–12.695
	45			–13.348

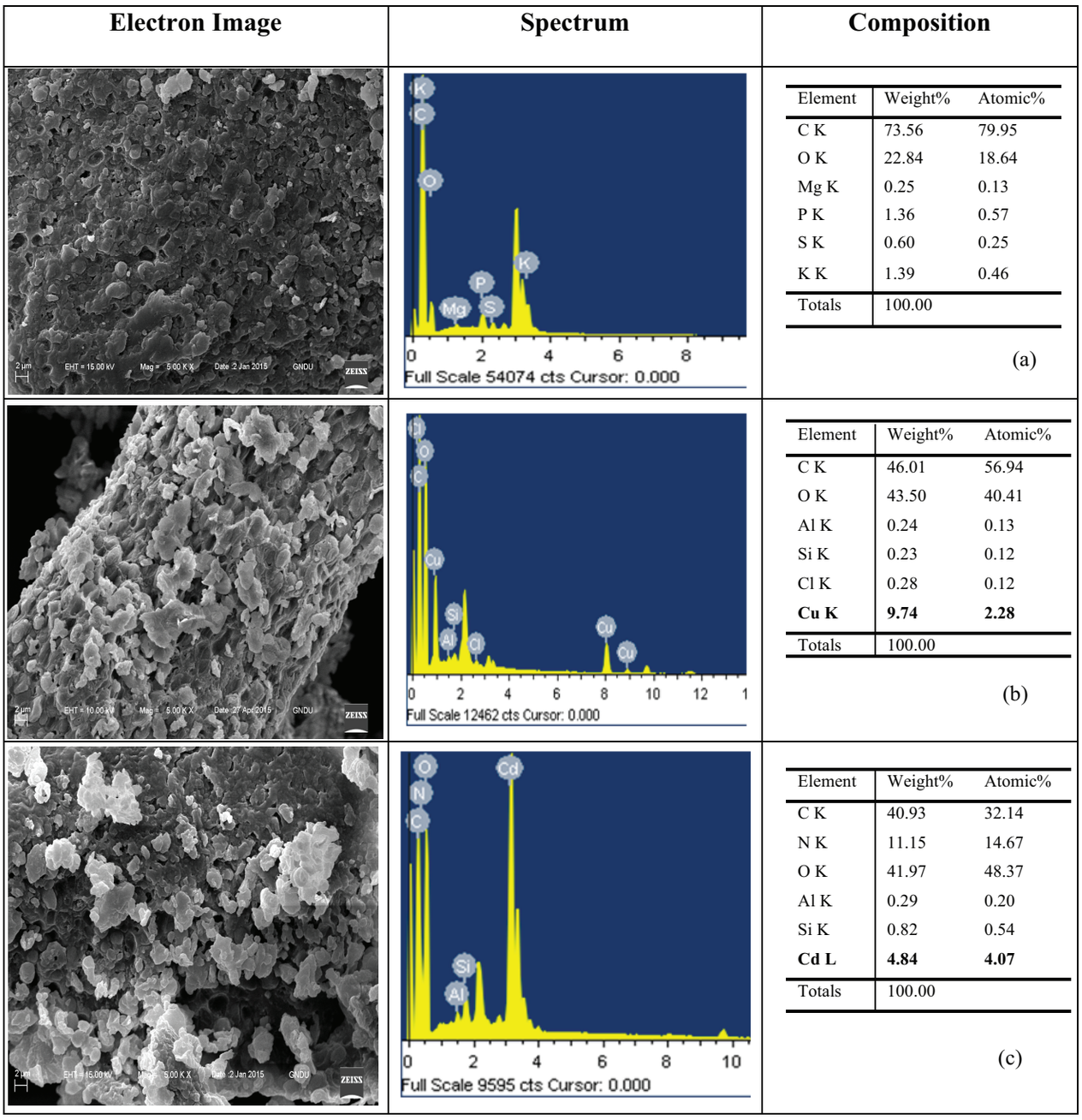
SEM studies revealed that copper and cadmium ions were accumulated due to sorption on the surface of *Saccharomyces cerevisiae*. Morphological changes were observed on the surface of *Saccharomyces cerevisiae* after biosorption of copper and cadmium (Fig. 4). Distinct peaks of copper and cadmium were observed on the EDX spectrum of *Saccharomyces cerevisiae* after biosorption which again confirmed biosorption of copper and cadmium ions on the surface of *Saccharomyces cerevisiae*. It was found that element composition in EDX spectrum of *Saccharomyces cerevisiae* was changed after biosorption process.

Percentage of nitrogen, carbon, hydrogen and sulfur was found to be 7.895, 61.4, 5.373 and 0.259 %, respectively before sorption process (Table 4). It was found that percentage of nitrogen and carbon was decreased where as hydrogen and sulfur content was almost diminished after biosorption of copper. Similarly, after the biosorption of cadmium onto *Saccharomyces cerevisiae* only sulfur content was diminished.

XRD spectrum of *Saccharomyces cerevisiae* before and after biosorption of copper and cadmium are shown in Fig. 5. It was observed from XRD spectrum of *Saccharomyces cerevisiae* (3a) did not show any noticeable sharp crystalline peak in the range of 10–80° which indicates amorphous nature of biosorbent. It can be seen in Fig. 3b that typical characteristic peaks of copper were observed as copper oxide chlorate (PDF: 350839; 2θ: 10.723, 16.204), copper ammine nitrite (PDF: 450025; 2θ: 12.916), copper nitrate hydrate (PDF: 852018; 2θ: 20.766, 25.956, 40.711, 58.601), copper nitrate hydrate (PDF: 240370; 2θ: 22.490, 24.675, 34.075, 36.977), copper nitrate hydroxide (PDF: 770148; 2θ: 43.711, 60.858) and copper hydroxide nitrate (PDF: 820059; 2θ:51.417, 64.384, 77.601) after biosorption of copper onto *Saccharomyces cerevisiae*. Similarly, in cadmium biosorbed *Saccharomyces cerevisiae*, the crystalline peak of cadmium acetate acetamide (PDF: 331610; 2θ: 12.431, 18.760), cadmium hydrate hydrazine carbonate (PDF: 771418; 2θ: 13.934, 21.890, 28.080, 30.354, 50.082, 52.879, 55.080, 64.115, 76.677), cadmium L-glutamate dihydrate (PDF: 492016; 2θ: 17.285) and cadmium diethylenetriamine nitrate (PDF: 461869; 2θ: 19.509) were observed.

A comparative study of flocculent strain of *Saccharomyces cerevisiae* for biosorption of copper and cadmium ions used in present study and other live forms of *Saccharomyces cerevisiae* already reported in literature has been made and summarized in Table 5. It is clear that biosorption capacity of flocculent strain of *Saccharomyces cerevisiae* for Cu (II) and Cd (II) were comparable [5] or moderately higher [5,22,35] than other live forms investigated in earlier studies.

The biosorption process for the removal of metal ions using living cells involves two mechanisms viz., passive and active. Passive mechanism (physical sorption) is indeed the first step of biosorption in which metal ions are biosorbed on the surface of live cells via different processes such as co-ordination, complexation and ion-exchange.



Element	Weight%	Atomic%
C K	73.56	79.95
O K	22.84	18.64
Mg K	0.25	0.13
P K	1.36	0.57
S K	0.60	0.25
K K	1.39	0.46
Totals	100.00	

(a)

Element	Weight%	Atomic%
C K	46.01	56.94
O K	43.50	40.41
Al K	0.24	0.13
Si K	0.23	0.12
Cl K	0.28	0.12
Cu K	**9.74**	**2.28**
Totals	100.00	

(b)

Element	Weight%	Atomic%
C K	40.93	32.14
N K	11.15	14.67
O K	41.97	48.37
Al K	0.29	0.20
Si K	0.82	0.54
Cd L	**4.84**	**4.07**
Totals	100.00	

(c)

Fig. 4. Scanning Electron Microscope-Energy-dispersive X-ray spectrometry (SEM EDX) analysis of (a) *Saccharomyces cerevisiae* before biosorption; (b) after the biosorption of copper and (c) cadmium.

During second step i.e. active mechanism (Physical and chemical sorption), metal ions penetrate into cell through cell membrane and get accumulated in the cytoplasm [38]. The present study clearly indicated that the process of sorption using *Saccharomyces cerevisiae* to biosorb copper and cadmium ions included both passive and active mechanism (Fig. 6).

During passive mechanism, a metabolism independent process, metal ions bound to the cell wall of *Saccharomyces cerevisiae* within 24 h by physical sorption, whereas, during active mechanism, metal ions entered into cytoplasm through cell membrane and the mechanism might have been metabolism dependent or independent. Finally, the accumulation of metal ions in the cytoplasm within next 24 h resulted the cell to enter into dead phase, therefore, biosorption was then independent of metabolic activity of the cells. The results are in line with earlier studies conducted using *Saccharomyces cerevisiae* [9,39–41].

Table 4
Percentage of nitrogen, carbon, hydrogen and sulfur before and after the biosorption process using *Saccharomyces cerevisiae*

Elements	*Saccharomyces cerevisiae*		
	Unloaded (%)	Cu-loaded (%)	Cd-loaded (%)
Nitrogen	7.895	2.475	4.634
Carbon	61.40	56.802	34.792
Hydrogen	5.343	0	3.343
Sulfur	0.259	0	0

4. Conclusion

Flocculent strain of *S. cerevisiae* possesses promising sorption capacity for the removal of copper and cadmium from aqueous solution. Biosorption equilibrium data was well fitted in Langmuir isotherm for biosorption of copper and cadmium with maximum biosorption capacity as 15.87 and 13.33 mg/g, respectively. Biosorption process for both metal ions was endothermic and spontaneous in nature and followed the pseudo second order kinetic model. Biosorption of copper and cadmium ions onto *S. cerevisiae* was confirmed using XRD and SEM-EDX analysis. The present study revealed that flocculent strain (MTCC-250)

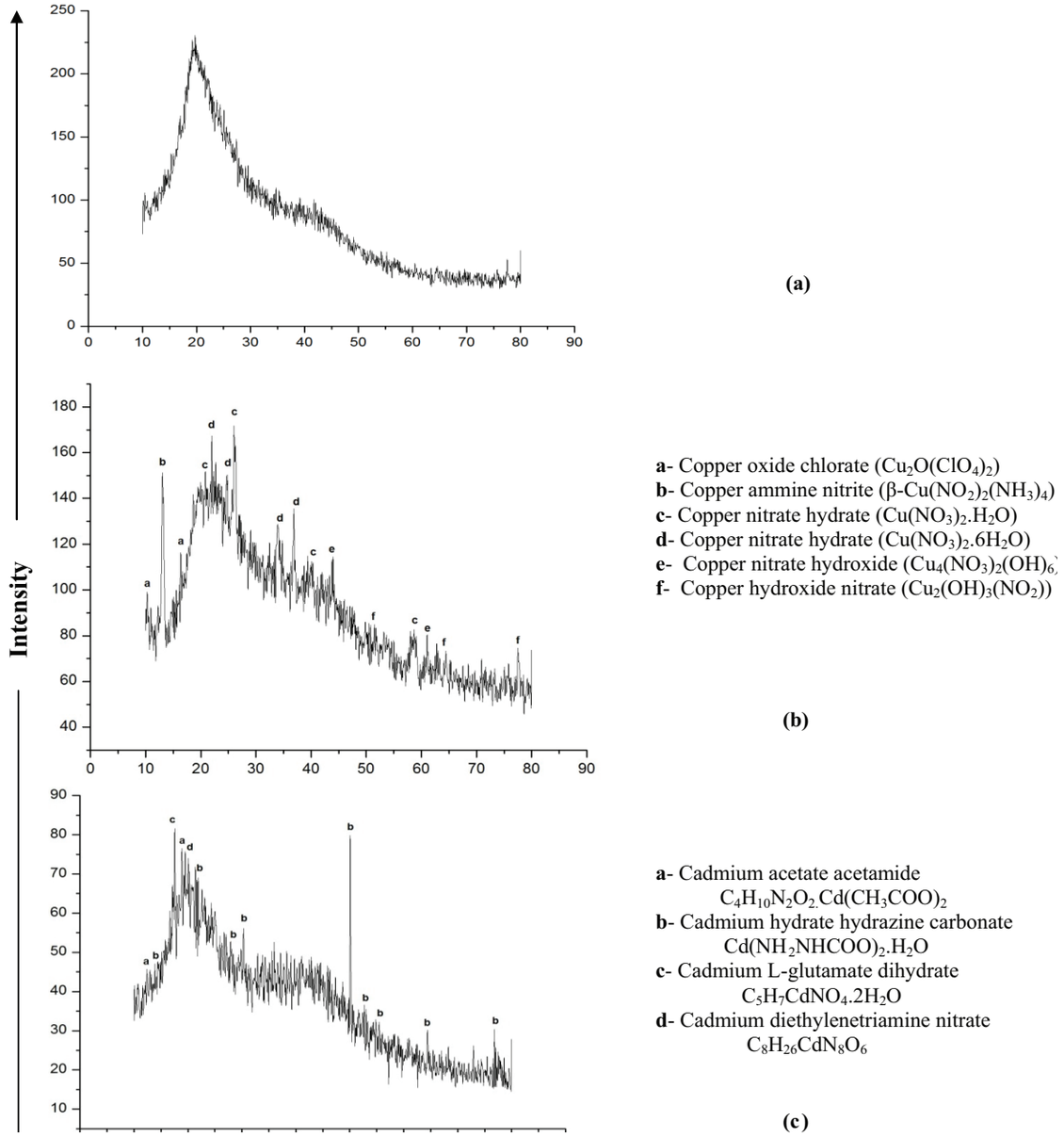


Fig. 5. XRD spectrum of (a) *Saccharomyces cerevisiae*, (b) copper loaded *Saccharomyces cerevisiae* and (c) cadmium loaded *Saccharomyces cerevisiae*

Table 5
Comparison of biosorption capacity of live forms of *Saccharomyces cerevisiae* for removal of heavy metal ions

S. No.	Types of *Saccharomyces cerevisiae*	Metal ions	Conditions	Maximum biosorption capacity (mg/g)	Isotherm used	Reference
1.	Live biomass	Cadmium (II) Copper (II)	Temp = 25°C , pH = 7.1, Dose = 4.07 g/L Contact time = 20 min, Shaking-150 rpm	16.63 4.17	Langmuir	Frahan and Khadam [5]
2.	DSM-1333 Strain	Cadmium (II)	Temp = 30°C , pH = 5.5, Dose = 0.4 g/L Contact time = 120 min, Shaking-150 rpm	16.18	Langmuir	Nagy et al. [22]
3.	Live biomass	Cadmium (II) Copper (II)	Temp = 25°C, pH = 5.0, Dose = 10 ml/100 ml, Contact time = 24 h, Shaking-7000 rpm for 5 min	2.368 1.347	Langmuir	Hamza et al. [35]
4.	Live biomass *S. cerevisiae* AUMC-3875	Cadmium (II)	Temp = 35°C , pH = 5.0, Dose = 1 g/l Contact time = 3 h, Shaking-120 rpm	12 .00	Langmuir	El-Sayed [37]
5.	Flocculent (MTCC-250) strain	Copper (II) Cadmium (II)	Temp = 25°C , pH = 7, Dose = 8 ml/l, Contact time = 48 h, Shaking-120 rpm	15.87 13.33	Langmuir/ Freundlich	Present work

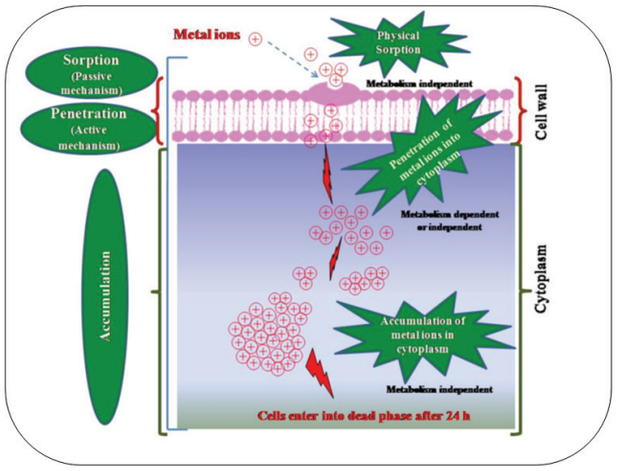


Fig. 6. Mechanism of biosorption of metal ions in *Saccharomyces cerevisiae*

of *S. cerevisiae* can be used as an effective biosorbent for removal of copper and cadmium from the industrial effluents and other water bodies.

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