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# Insight into Biosoprtion Equilibrium, Kinetics and Thermodynamics Studies of Atrazine on to *Pichia Kudriavzevii* Atz-EN-01 and *Cryptococcus laurentii* Atz-EN-02

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## ABSTRACT

This study deals with the biosorption potential of two dead yeast *viz Pichia kudriavzevii* Atz-EN-01 and *Cryptococcus laurentii* Atz-EN-02 for atrazine removal from aqueous solution. The influence of operational parameters *viz* solution pH, biomass dosage, temperature, initial atrazine concentration and contact time on atrazine biosorption were studied in batch experiments. Isotherm models, kinetic models and thermodynamic parameters were analyzed to describe the adsorptive behaviour of the two yeast biosorbents. The results showed that *P. kudriavzevii* Atz-EN-01 exhibited the highest atrazine removal efficiency of 90% at pH 5.0. The adsorption isotherm data fitted well to the Langmuir isotherm with monolayer adsorption capacity of 74 mg/g for Atz-EN-01 and 53.55 mg/g for Atz-EN-02. The kinetic data indicated that biosorption of atrazine agreed well with the pseudo second order kinetic model and the intraparticle diffusion was not the rate limiting step for atrazine biosorption. The thermodynamic analysis showed spontaneous and exothermic nature of the biosorption process. Based on the Fourier transform infrared analysis, amide and carboxyl groups were found to be responsible for atrazine biosorption In conclusion, biomass of Atz-EN-01 was found to be a better biosorbent for atrazine removal as compared to the biomass of Atz-EN-02.

**Keywords:** Atrazine; Biosorption; Isotherm models; Intraparticle diffusion.

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## INTRODUCTION

Among the pesticides produced, atrazine, a chlorinated pesticide takes the front seat by production as well as usage [1]. The sources of atrazine pollution include agricultural runoff, aerial transport and pesticide industrial effluent. There are reports on contamination of surface waters, ground waters and soils by atrazine [2]. Due to its various toxicity properties, its permissible limit in drinking water has been restricted to 2 µg/L by World Health Organization [3] and maximum contaminant level 3 µg/L by USEPA [4]. But due to its high rate of usage and high persistence in neutral environment, high amount of atrazine i.e. 22 mg/L was detected in ground water [5, 6]. Domestic wastewater can also be contaminated by atrazine [7].

There are a number of technologies available for the treatment of atrazine contaminated water. Most commonly employed chemical methods for remediation of atrazine bearing wastewaters are photolysis, hydrolysis, dehalogenation and oxygenation. However, each of the methods has some disadvantages in practical applications [8].

Biosorption appears to be a low cost and non toxic approach having regeneration capability and high efficiency for pollutant uptake [8]. It is an energy independent, growth independent and surface binding phenomenon. In most of the cases, dead cells are more efficient in technical and economical aspect compared to live cells.

So far, no report is available in literature on atrazine biosorption using yeast as adsorbent. In this paper, we present the data on biosorption of atrazine using dead biomass of yeast strains *viz. Pichia kudriavzevii* Atz-EN-01 and *Cryptococcus laurentii* Atz-EN-02. The influence of the system variables including particle size, pH, biosorbent dosage, initial atrazine concentration on biosorption was studied. Three isotherm adsorption models *viz*. Langmuir, Freundlich and Temkin were applied to the experimental data to describe the biosorption equilibrium between atrazine and the dead yeast biomass. The experimental data were analyzed using the pseudo first order and pseudo second order kinetic models. In addition, intra particle diffusion model was done to evaluate the diffusion mechanism of atrazine. Thermodynamic parameters and the activation energy of the biosorption of atrazine by yeast biomass were also calculated.

## MATERIALS AND METHODS

### Chemicals

Atrazine (98.8%) and all the other reagents used in this present study were purchased from Sigma Aldrich, Bangalore, India and were used without further purification. Stock solution was prepared by dissolving 1g of atrazine in 1L of double distilled water. Atrazine solutions with different concentrations were prepared from this stock. For adjusting pH of the medium, 0.1 N solutions of NaOH and HCl were used.

**Isolation and Identification of Yeast**

Atrazine contaminated soil samples were collected from agricultural field located in the outskirts from Vellore district, Tamil Nadu, India which has a long history of atrazine usage. Yeast species were isolated from the contaminated soils by standard enrichment method. The partial 18S rRNA and ITS genes of the isolated yeasts were PCR amplified with the following forward and reverse primers: UL18F: 5' - TGTACACACCGCCCGTC - 3' and UL28R: 5' - ATCGCCAGTTCTGCTTAC -3'. PCR products containing the amplified gene sequences were purified using clean GeNei$^{TM}$ kit (Bangalore) and sequenced using the above primers, along with UL620R: 5' - TGGTCCGTGTTTCAAGA -3' primer. The sequencing results of the isolated yeasts were then submitted to GenBank under the accession numbers KC886644 and KC920643. The nucleotide sequence similarities were determined by a BLAST search through the National Centre for Biotechnology Information Internet site. The yeast isolate submitted under the accession number KC886644 was closely related to the yeast species *Pichia kudriavzevii* (99% homology with *Pichia kudriavzevii* strain NRRL Y-5396, EF 550222.1). The other isolated yeast (Accession number KC920643) exhibited close relation with yeast species *Cryptococcus laurentii* (99% homology with *Cryptococcus laurentii* strain IMUFRJ51993, FN428900.1). Therefore, the isolated yeasts designated as *Pichia kudriavzevii* Atz-EN-01 (KC886644) and *Cryptococcus laurentii* Atz-EN-02 (KC920643) were used for the experiments.

**Preparation of Biosorbent**

The two yeast strains *viz Pichia kudriavzevii* Atz-EN-01 and *Cryptococcus laurentii* Atz-EN-02 were cultivated in mineral salt medium of the following composition (g/L): $K_2HPO_4$, 0.4; $KH_2PO_4$, 0.2; NaCl, 0.1; $MgSO_4.7H_2O$, 0.5; $MnCl_4$, 0.01; Fe $(SO_4)_3$, 0.01; $Na_2MoO_4$, 0.01 and atrazine, 0.25; pH 6 at 30°C for 5 days. The yeast biomass was harvested by centrifugation at 5000 rpm for 5 mins and subjected to drying at 40°C. For the biosorption studies, the dried yeast biomass was then finely powdered and sieved through standard sieves to constant sizes.

**Batch Biosorption Process**

All the experiments were carried out using the dried desired yeast biomass dosage in 100 mL of conical flask containing atrazine solution, to elucidate the optimum values of the experimental parameters viz. particle size of the biosorbent (150- 600µm), pH (2-9), biosorbent dosage (1-5 g/L) temperature (10-35°C), initial atrazine concentration (50-350 mg/L) and contact time (15- 540 min). The experiments were carried out in dark to prevent photodegradation of atrazine. The solutions were agitated for desired time at 120 rpm and the samples were withdrawn at desired time and subjected to centrifugation at 5,000 rpm for 5 min. The residual concentrations of the supernatant were analysed by gas chromatography (NUCON, Model 5765). Control experiments were performed under identical conditions, but without any biomass. All experiments were done in duplicate.

The amount of atrazine biosorbed was calculated from the differences between the atrazine quantity added to the biomass and the atrazine content of the supernatant using the mass balance equation:

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$$q_e = \frac{C_0 - C_f}{M} \times V \qquad (1)$$

Where $q_e$ is the equilibrium uptake (mg/g), $C_0$ is the initial atrazine concentration (mg/L), $C_f$ is the equilibrium atrazine concentration (mg/L), V is the volume of the aqueous phase (L) and M is the quantity of the biomass (g).

The removal percentage (E, %) was calculated using the equation:

$$E\ (\%) = \frac{C_0 - C_f}{C_0} \times 100 \qquad (2)$$

**Equilibrium and Kinetic Modelling**

Equilibrium sorption and kinetic studies were conducted by varying the initial atrazine concentration from 50-350 mg/L with biosorbent dosage of 3 g/L. The pH was constantly measured and adjusted using 0.1 N NaOH or HCl. The samples were separated and determined for their residual atrazine concentration using gas chromatography. From the experimental data, different sorption models i.e. Langmuir, Freundlich and Temkin isotherm models were evaluated for applicability. Isotherm constants and regression coefficient square value ($R^2$) were determined from the sorption models. The kinetics of atrazine biosorption on yeast biomasses was described with different kinetic models, *viz*. pseudo first order, pseudo second order model and intraparticle diffusion experiments. Using equilibrium constants with temperature, thermodynamic parameters such as Gibbs free energy change ($\Delta G^\circ$), enthalpy change ($\Delta H^\circ$) and entropy change ($\Delta S^\circ$) were estimated.

**Fourier Transform Infrared (FT-IR) Spectral Analysis**

Infrared spectra were recorded on an Avatar 330 model (ThermoNicolet Co., USA) FT-IR spectrometer. For IR studies, 5 mg of biosorbent (native and atrazine interacted) was encapsulated in 400 mg of KBr. Translucent discs 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.

**Scanning Electron Microscopy (SEM) Analysis**

The surface morphology of native and atrazine interacted yeast biomass samples were recorded using scanning electron micro-scope (SEM) (Stereo Scan LEO, Model-400).

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## RESULTS AND DISCUSSION

### Effect of Particle Size on Atrazine Removal

Removal of atrazine is related with surface area of adsorbent directly. So, the particle size of adsorbent is one of the important factors affecting the atrazine removal process (9). Removal of atrazine was studied at three different particle sizes (150 -300 µm, 300-425 µm and 425-600 µm) of yeast biomass. The results are shown in Figure 1a. It was found that atrazine removal decreased with increasing particle size of the adsorbent. Maximum atrazine removal efficiency was noted at 150 -300 µm. This was because the increase in the total surface area of smaller particles provided more sorption sites for atrazine.

### Effect of Initial pH on Atrazine Removal

The effect of initial pH on the removal of atrazine by yeast species was studied at pH 2.0-9.0. Figure 1b showed that atrazine removal efficiency increased along with the increase of pH of the adsorbate solution for both the yeast strains. Maximum atrazine removal efficiency was observed at pH 5.0. An increase or decrease from this optimum pH resulted in reduction of the atrazine removal efficiency. This may be due to the overall surface charge on yeast biomass becomes positive at pH<3 whereas, strong repulsive force prevails at higher pH [10]. These results were in accord with the results reported by Rao and Viraraghavan [11], in which the biosorption of phenol from an aqueous solution was evaluated using *A. niger* as adsorbent and maximum removal of phenol was obtained at an initial pH 5.1. They also reported that the electrostatic forces between the charged fungal surface and phenol played an important role in the biosorption of phenol.

### Effect of Biosorbent Dosage on Atrazine Removal

The effect of different doses on atrazine removal efficiencies of biosorbents were carried out, and the results have been presented in Figure 1c. The amount of biosorbent was varied from 1 to 5 g/L, while all the variables such as particle size, pH, temperature , initial atrazine concentration, and contact time were kept constant. It could be seen the atrazine removal efficiency of yeast biomass increased with the increase in biosorbent dosage and 3 g/L was found to be the optimum dosage for both the strains. This increase in atrazine removal efficiency is due to the availability of higher number of solutes (atrazine) per unit mass of adsorbent, i.e., higher solute/adsorbent ratio. Similar result were reported by other workers [12, 13] in the adsorption of 2, 4, 6-trichlorophenol from aqueous solution by *Acacia leucocephala* bark.

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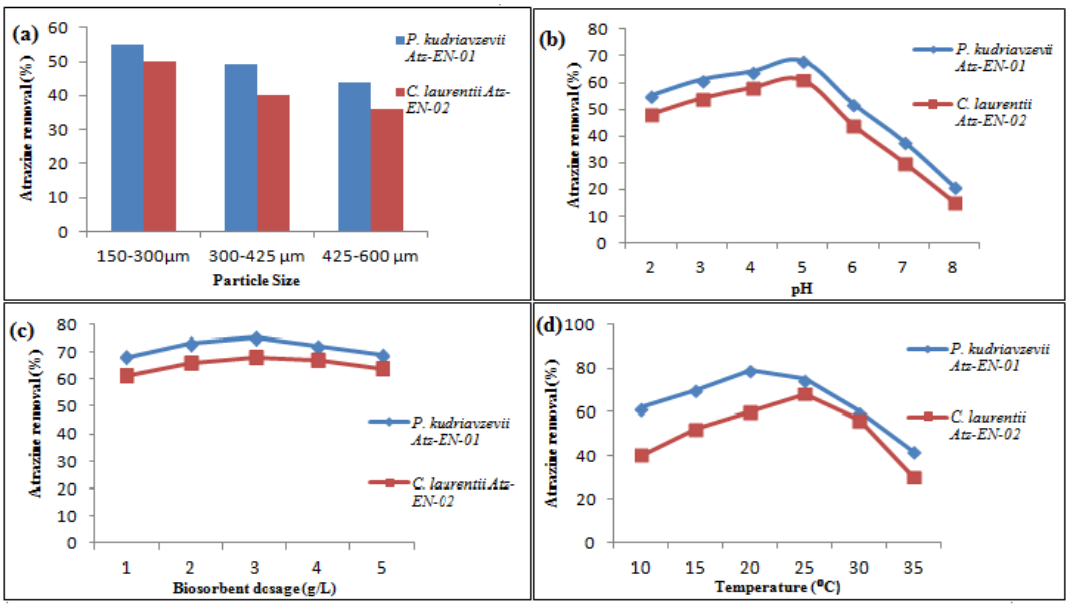


**Figure 1: Effects of (a) Particle Size (pH 2, Biomass Dosage 1 g/L, Temperature 25° C); (b) pH (Particle Size 150-300µm, Biomass Dosage 1 g/L, Temperature 25° C); (c) Biomass Dosage (Particle Size 150-300 µm, pH 5, Temperature 25° C); (d) Temperature (particle size 150-300 µm, pH 5, Biomass Dosage 3 g/L) on Atrazine Removal Using Dead Yeast as Biosorbents.**

## Effect of Temperature on Atrazine Removal

Temperature is a crucial parameter in biosorption reactions as thermodynamic properties of the process can be calculated on effect of temperature basis [14]. Temperatures ranging from 10, 15, 20, 25, 30 and 35°C were selected in this study. The effect of temperature on the removal of atrazine by yeast biomass at an initial concentration of 50 mg/L was shown in Figure 1d. The optimum temperature, at which the atrazine removal by yeast biomass was the highest, was 25°C in the studied temperature range. The atrazine removal decreased with increase in temperature as the molecules adsorbed earlier on a surface tend to dissolve from the surface [14].

## Effect of initial Atrazine Concentration on Atrazine Removal

The effect of initial concentration on the atrazine removal efficiencies of yeast biomass from aqueous solutions was carried out. All the other parameters like pH, biosorbent dosage, temperature and contact time were kept constant. Figure 2a showed the relation between atrazine removal efficiency and initial atrazine concentration, which indicated that as atrazine concentration increased the atrazine removal efficiency of the yeast strains also increased. Maximum sorption capacity of atrazine was noted at 250 mg/L and 200 mg/L in case of *P. kudriavzevii* Atz-EN-01 and *C. laurentii* Atz-EN-02 respectively. The initial concentration provides an important driving force to overcome all mass transfer resistances of adsorbate between the aqueous solid phase and therefore increases the rate at which adsorbate

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molecules pass from the bulk solution to then adsorbent surface [15, 16].The rapid atrazine removal feature was in agreement with the results of Wu and Yu [17].

**Effect of Contact Time on Atrazine Removal**

Figure 2b showed that the atrazine removal efficiencies of the biosorbents sharply increased with the increased in time and attained equilibrium in 240 minutes for both the adsorbents. The atrazine removal efficiencies remained constant after an equilibrium time of 240 minutes, which indicated that the removal tends towards saturation. The rate of removal is higher in the beginning due to large surface area available of the biosorbent. After the capacity of the adsorbent gets exhausted, i.e. at equilibrium, the rate of atrazine removal is controlled by the rate at which the absorbate is transported from the exterior to the interior sites of the biosorbent particles [18].

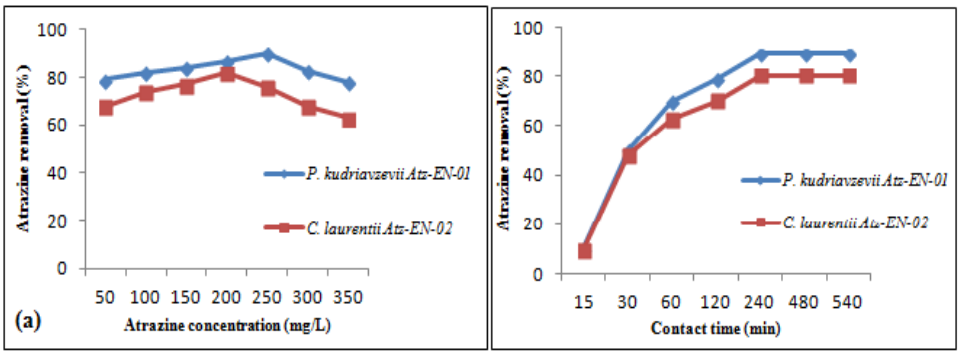


**Figure 2: Effects of (a) Initial Atrazine Concentration (Particle Size 150-300 µm, pH 5, Biomass Dosage 3 g/L, Temperature 25° C); (b) Contact Time (Particle Size 150-300 µm, pH 5, Biomass Dosage 3 g/L, Temperature 25° C, Initial Atrazine Concentration 250 mg/L for *P. kudriavzevii* Strain Atz-EN-01 and 200 mg/L for *C. laurentii* Atz-EN-02) on Equilibrium Sorption Capacity of Atrazine by Yeast Biosorbents.**

**Equilibrium Modelling**

Analysis of equilibrium data is important for developing a model that can be used for designing the adsorption system. Various isotherm equations have been used for equilibrium modelling of biosorption systems [19, 20]. Three classical adsorption models i.e., Langmuir, Freundlich and Temkin isotherms were used to describe the relationship between the amount of atrazine adsorbed and its equilibrium concentrations in solutions.

Langmuir equation is valid for monolayer sorption on to a surface with a finite number of identical sites and its expression is given by (Eq. 3)

$$q_{eq} = \frac{q_{\max} b C_{eq}}{1 + b C_{eq}} \qquad (3)$$

Where $q_{eq}$ (mg/g) and $C_{eq}$ (mg/L) are amount of absorbed atrazine per unit weight of biomass and unadsorbed atrazine concentration in solution at equilibrium respectively. $q_{max}$ (mg/g) is the maximum amount of atrazine per unit weight of biomass required to form a

complete monolayer on the surface bound at high $C_{eq}$ and b is a constant. $q_{max}$ and b are evaluated from the linear plot of the logarithmic equation Eq. 4

$$\frac{1}{q_{eq}} = \frac{1}{q_{\max}} + \frac{1}{bq_{\max}C_{eq}} \qquad (4)$$

The linear plot of $1/q_{eq}$ against $1/C_{eq}$ for both the yeast biosorbents showed that the adsorption obeyed the Langmuir model (Figure 3a). Langmuir constants $q_{max}$ and b were determined from the slope and intercept of the plot and presented in Table 1. The $R^2$ values (0.9613 and 0.9398) for strains *P. kudriavzevii* Atz-EN-01and *C. laurentii* Atz-EN-02 suggested that the Langmuir isotherm provided a good fit to the isotherm data. The Freundlich equation based on sorption on a heterogeneous surface is given below as Eq. (5):

$$q_e = K_F C_e^{1/n} \qquad (5)$$

Where $K_F$ and n are Freundlich constants, whereas $K_F$ and n are indicators of adsorption capacity and adsorption intensity of the sorbents respectively (9, 21).
Eq. 5 can be linearized in logarithmic form as Eq. 6

$$\log q_{eq} = \log K_{F+} \frac{1}{n} \log C_{eq} \qquad (6)$$

The values of Freundlich constants were calculated from the intercepts and slope of the plot (Figure 3b) and listed in Table 1. The $R^2$ values (0.8126 and 0.8498) for *P. kudriavzevii* Atz-EN-01and *C. laurentii* Atz-EN-02 are lower than Langmuir isotherm.

Temkin isotherm assumes that the heat of adsorption of all the molecules in a layer decreases lineraly with surface coverage of sorbate-adsorbate interactions. This adsorption is characterized by a uniform distribution of binding energies. Temkin isotherm has been represented by the following Eq. 7

$$q_{eq} = \frac{RT}{b}[\ln(AC_{eq})] \qquad (7)$$

Eq. 7 can be represented in its linear form as Eq. 8.

$$q_{eq} = B\ln A + B\ln C_{eq} \qquad (8)$$

Where B=RT/b, T is the absolute temperature and R is the universal gas constant, A is the equilibrium binding constant and the constant B is related to the heat of adsorption.

The values of the isotherm constants A and B were determined from Figure 3c, as listed in Table 1.The $R^2$ values (0.8701 and 0.8937) for *P. kudriavzevii* Atz-EN-01 and *C. laurentii*

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Atz-EN-02 were found to be lower than the Langmuir model. On the basis of the linear square regression correlation coefficient $R^2$ values, the best equilibrium model was represented by Langmuir isotherm which indicated that atrazine adsorption by dead yeast biomass followed monolayer adsorption system.

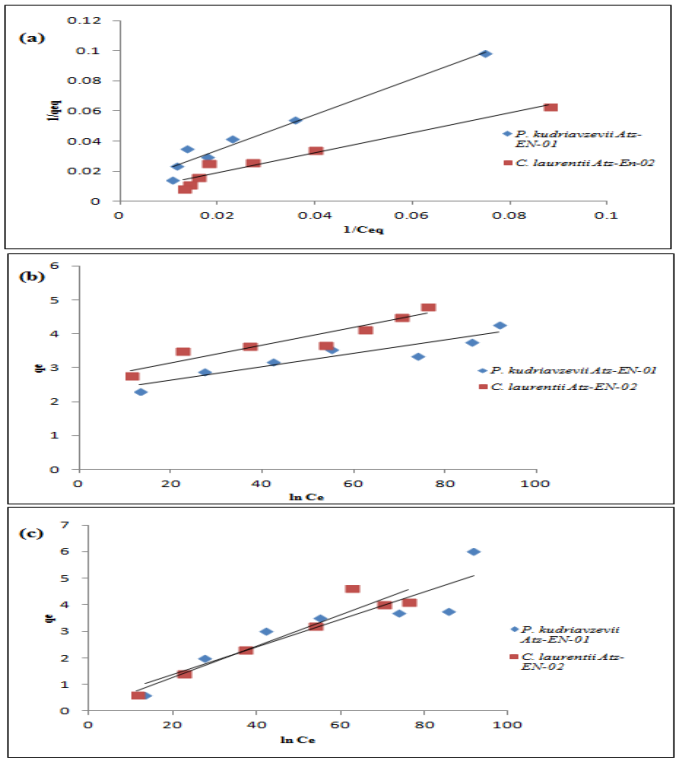


**Figure 3: Adsorption Isotherms of (a) Langmuir (b) Freundlich and (c) Temkin at Different Concentrations of Atrazine by Dead Yeast Biomass (Particle Size 150-300 µm, pH 5, Biomass Dosage 3 g/L, Temperature 25° C).**

**Table 1. Isotherm Constants for Atrazine on Dried Biomass of *P. kudriavzevii* Atz-EN-01 and *C. laurentii* Atz-EN-02 (pH 5, Biomass Dosage 3 g/L, Initial Atrazine Concentration 50-350 mg/L).**

| S.No. | Isotherm Model | Parameters | Biosorbents | |
|---|---|---|---|---|
| | | | *P. kudriavzevii* Atz-EN-01 | *C. laurentii* Atz-EN-02 |
| 1 | Langmuir | $q_{max}$ (mg/g) | 74.071 | 53.601 |
| | | b | 0.0113 | 0.0111 |
| | | $R^2$ | 0.9613 | 0.9398 |
| 2 | Freundlich | $K_f$ (mg/g) (L/mg)$^{1/n}$ | 1.7931 | 1.0701 |
| | | N | 1.1651 | 1.1240 |
| | | $R^2$ | 0.8126 | 0.8498 |
| 3 | Temkin | A (l/g) | 1.1901 | 1.1341 |
| | | B | 16.340 | 15.761 |
| | | $R^2$ | 0.8416 | 0.9253 |

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**Adsorption Kinetics**

To evaluate the sorption kinetics of atrazine, two kinetic models *viz* Pseudo first order and second order models were used to fit the experimental data at different initial concentration at pH 5 and temperature 25°C. The pseudo first order rate expression of Lagergren model is generally expressed as follows

$$\log(q_{eq} - q_t) = \log q_e - \frac{K_1}{2.303} t \qquad (9)$$

Where $K_1$ (1/min), is the rate constant, $q_e$ (mg/g) is the amount of solute adsorbed on the surface at equilibrium and $q_t$ (mg/g) is the amount of solute adsorbed at any time. From the plot log ($q_e$- $q_t$) against t, value of $K_1$ for both the yeast biosorbents was calculated. The first order equation of Lagergren for *P. kudriavzevii* Atz-EN-01 (Figure 4a) and *C. laurentii* Atz-EN-02 (Figure 4c) does not fit well the whole range of contact time and is generally applicable over the initial stage of the adsorption processes. The parameters indicated that this model had failed to estimate $q_{eq}$ since the experimental values of $q_{eq,\ exp}$ differed from estimated $q_{eq,\ cal}$ (Table 2). Therefore, the pseudo second order kinetic model [22] as shown in Eq. 10 was used to study the kinetics of the present system. Figure 4b and Figure 4d represents pseudo second order kinetic model for both the yeast strains.

$$\frac{t}{q_t} = \frac{1}{K_2 q^2_{eq}} + \left(\frac{1}{q_{eq}}\right) t \qquad (10)$$

Where $K_2$ ( g/mg min) is the second-order rate constant. From the slope and intercepts of the plot $t/q_t$ versus t, $q_{eq}$ and $K_2$ were calculated. The initial sorption rate h (mg/g min) for both the yeast species were calculated from second order rate constant $K_2$, where t tends to 0.

$$h = K_2\, q^2_{eq} \qquad (11)$$

Table 2 represented the pseudo second order constants $K_2$, calculated h values and corresponding linear regression correlation coefficients $R^2$ values for both the yeast species. The $R^2$ values were found to be in the range of 0.91-1.0 for both the yeast strains. Moreover, the data showed less variations between the calculated ($q_{eq,\ cal}$) and experimental ($q_{eq,\ exp}$) values. The high agreement between the calculated and experimental $q_{eq}$ values along with the high correlation coefficient values indicated that pseudo second order kinetics were best fitted over the other model for the adsorption of atrazine on the yeast biosorbents. This suggested that adsorption process was controlled by chemisorption. The values of initial adsorption rates, h for both the yeast species increased with increase in initial atrazine concentration. This might be due to the increase in the driving force for mass transfer, allowing more atrazine molecules to reach the surface of the adsorbents in a shorter period of time [22].

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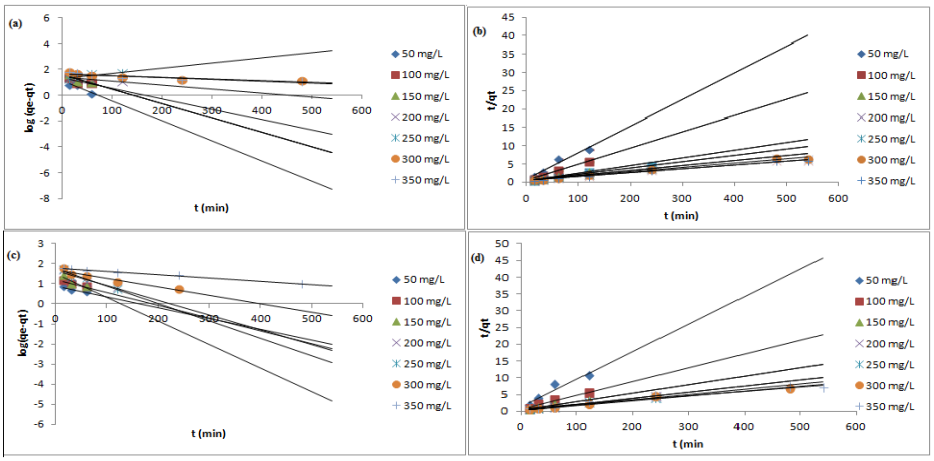


**Figure 4: Linearized Pseudo First Order and psEudo Second Order Kinetic Models for Atrazine Sorption by *P. kudriavzevii* Atz-EN-01 ( a & b) and *C. laurentii* Atz-EN-02 (c & d) at Different Initial Concentrations (particle size 150-300 µm, pH 5, Biomass Dosage 3 g/L, Temperature 25° C).**

**Table 2. Comparison of the Pseudo First Order, Pseudo Second order Adsorption Rate Constants, Calculated and Experimental $q_{eq}$ Values Obtained at Different Initial Atrazine Concentrations (particle size 150-300 µm, pH 5, Biomass Dosage 3 g/L, Initial Atrazine Concentration 50-350 mg/L.**

| Biosorbents | Initial atrazine concentration (mg /L) | $q_{eq\ exp.}$ (mg/g) | Pseudo first order kinetic model | | | Pseudo second order kinetic model | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | $K_{1\ (1/min)}$ | $q_{eq,cal.}$ (mg/g) | $R^2$ | $K_2$ (g/mg/min) $10^4$ | $q_{eq,\ cal}$ (mg/g) | $R^2$ | $h_0$ |
| *P. Kudriavzevii* Atz-EN-01 | 50 | 13.16 | 0.0359 | 12.445 | 0.876 | 0.00600 | 13.77 | 0.947 | 1.137 |
| | 100 | 21.33 | 0.0204 | 23.380 | 0.701 | 0.00520 | 22.47 | 0.993 | 1.276 |
| | 150 | 42.0 | 0.0260 | 40.878 | 0.697 | 0.00044 | 42.61 | 0.995 | 1.365 |
| | 200 | 55.0 | 0.0007 | 25.130 | 0.933 | 0.00036 | 56.14 | 0.984 | 1.580 |
| | 250 | 74.0 | 0.0009 | 20.600 | 0.810 | 0.00031 | 74.62 | 0.999 | 1.753 |
| | 300 | 85.6 | 0.0029 | 46.550 | 0.830 | 0.00026 | 85.47 | 0.985 | 1.904 |
| | 350 | 92.15 | 0.0035 | 47.640 | 0.787 | 0.00026 | 85.47 | 0.985 | 2.228 |
| *C. laurentii* Atz-EN-02 | 50 | 11.35 | 0.0124 | 7.941 | 0.899 | 0.00470 | 12.19 | 0.9171 | 0.704 |
| | 100 | 22.22 | 0.0147 | 16.59 | 0.94 | 0.0025 | 24.39 | 0.96 | 1.52 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | | 8 | 6 | | 97 | 2 |
| 150 | 37.03 | 0.0269 | 31.62 | 0.786 | 0.00217 | 39.68 | 0.9876 | 3.410 |
| 200 | 53.55 | 0.0170 | 43.65 | 0.873 | 0.00167 | 54.94 | 0.9851 | 5.041 |
| 250 | 62.23 | 0.0200 | 57.54 | 0.932 | 0.00068 | 64.51 | 0.9618 | 2.865 |
| 300 | 70.24 | 0.0096 | 46.77 | 0.934 | 0.00052 | 71.42 | 0.9857 | 2.651 |
| 350 | 76.01 | 0.0039 | 60.225 | 0.993 | 0.00024 | 76.33 | 0.9613 | 1.411 |

**Thermodynamics**

The Gibbs energy, enthalpy and entropy (ΔG, ΔH, ΔS), for the adsorption process were obtained from the experiments carried out at different temperatures using the following Eq. 12.

$$\log\left(\frac{q_e}{C_e}\right) = \frac{\Delta S}{2.303R} - \frac{\Delta H}{2.303RT} \qquad (12)$$

$$\Delta G = \Delta H - T\Delta S \qquad (13)$$

Where $q_e/C_e$ is called the adsorption affinity, which is the ratio of $q_e$, the amount adsorbed 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 [23, 24].

In order to describe the thermodynamic behaviour of biosorption of atrazine onto both the yeast biomass, thermodynamic parameters including the change in free energy (ΔG), enthalpy (ΔH) and entropy (ΔS) were calculated from the equations given earlier (Eq. 12) and ( Eq. 13). The results on effect of temperature indicated that the maximum uptake of atrazine on both the yeast species were at 25°C. The value of ΔG obtained at 25°C (−0.042 kJ/mol for *P. kudriavzevii* Atz-EN-01) and (-0.010 kJ/ mol for *C. laurentii* Atz-EN-02) indicated maximum spontaneity whereas positive ΔG values at other temperatures apart from 25°C indicated less spontaneity resulting in decreased atrazine uptake. The values of ΔH and ΔS were determined from the slope and intercept of plot of log $q_e/C_e$ vs. 1/T as shown in Figure 5. for both the yeast strains. The values of ΔH and ΔS for sorption of atrazine on *P. kudriavzevii* Atz-EN-01were found to be -0.004 kJ/mol and −1.149 kJ/mol/K whereas for *C. laurentii* Atz-EN-02 the values of ΔH and ΔS were found to be -0.006 kJ/mol and 1.719 kJ/mol/K respectively. The negative values of ΔH indicated the exothermic nature of the sorption process and the negative value of ΔS suggested a decrease in the randomness at the solid/ solution interface at 25°C.

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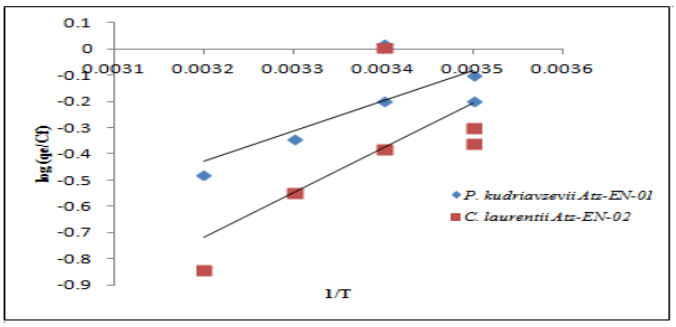


**Figure 5: The Plot of Log ($q_e/C_e$) versus 1/T for Adsorption of Atrazine at Different Temperatures (Particle Size 150-300 µm, pH 5, Biomass Dosage 3 g/L, Contact Time 240 mins).**

## Intraparticle Diffusion

The kinetic results can be used to test the presence or absence of intraparticle diffusion and to determine whether intraparticle diffusion is the rate-limiting step for biosorption. The linear plots (Figure 6) demonstrated the presence of intraparticle diffusion in the biosorption process of atrazine by both yeast biosorbents. All the curves had same features, i.e., an initial curve portion, followed by a linear portion and later a plateau. This indicated that more than one mode of sorption were involved in the sorption of atrazine. The initial curve portion was attributed to the boundary layer sorption, the linear portion to the intraparticle diffusion and the plateau to the equilibrium. If intraparticle diffusion is involved in the sorption process [25, 26], a plot of adsorption uptake versus the square root of time will thus result in a linear relationship and the intraparticle diffusion should be the rate-controlling step if the line passes through the origin. Although in Figure 6 there was a linear relationship over a period of time, they did not pass through the origin, suggesting that intra-particle diffusion was present, but not the only rate-controlling step, and that some other mechanisms might be involved in the biosorption of atrazine by the yeast biomass. The rate constants for the intraparticle diffusion $K_{id}$ are listed in Table 3.

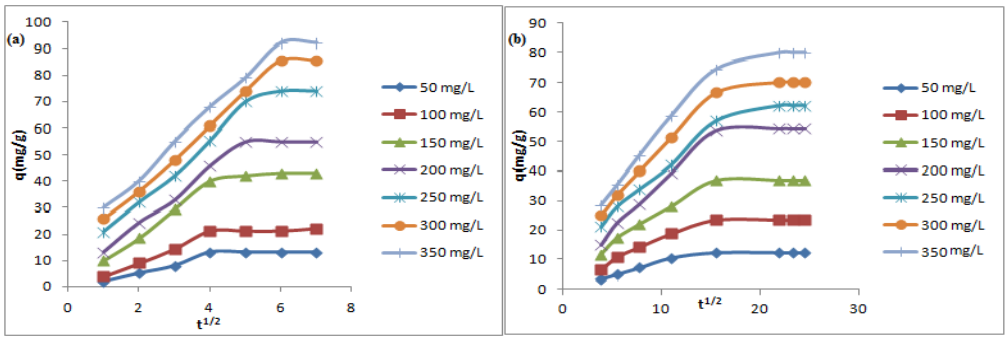


**Figure 6: Intraparticle Diffusion Plots for Adsorption of Atrazine by *P. kudriavzevii* Atz-EN-01 (a) and C. *laurentii* Atz-EN-02 (b) at Different Temperatures (particle size 150-300 µm, pH 5, Biomass Dosage 3 g/L, Initial Atrazine Concentration 50-350 mg/L; Contact Time 240 mins).**

**Table 3. Intraparticle Diffusion Model Constants and Correlation Coefficients for Adsorption of Atrazine on Dried Yeast Biomass (Particle Size 150-300 µm, pH 5, Biomass Dosage 3 g/L, Initial Atrazine Concentration 50-350 mg/L , Contact Time 240 mins).**

| Initial Atrazine Concentration (mg/L) | *P. kudriavzevii* Atz-EN-01 | | | *C. laurentii* Atz-EN-02 | | |
|---|---|---|---|---|---|---|
| | $K_{id}$ | C | $R^2$ | $K_{id}$ | C | $R^2$ |
| 50 | 1.9481 | 1.8970 | 0.815 | 0.401 | 3.7590 | 0.810 |
| 100 | 3.0891 | 3.8951 | 0.835 | 0.737 | 7.5370 | 0.833 |
| 150 | 5.7340 | 9.3280 | 0.854 | 1.155 | 12.024 | 0.862 |
| 200 | 7.4391 | 10.457 | 0.903 | 1.837 | 14.202 | 0.881 |
| 250 | 9.6820 | 13.886 | 0.949 | 1.983 | 18.011 | 0.940 |
| 300 | 10.843 | 16.114 | 0.976 | 2.162 | 22.650 | 0.913 |
| 350 | 11.241 | 20.221 | 0.975 | 2.468 | 22.360 | 0.920 |

The results of intraparticle diffusion showed an increase in the rate constant for intraparticle diffusion with increasing initial atrazine concentration. This is in consistent with the fact that diffusion needs some energy to overcome the mass transfer resistance. The presence of the intraparticle diffusion implied that both pore diffusion and kinetic resistances might affect the adsorption rate. Similar trend of result was reported by Wu and Yu [17] in the biosorption of phenol and chlorophenols from aqueous solutions by fungal mycelia.

**FT-IR Analysis**

Infrared spectra can yield valuable information regarding the chemical groups possessed by the biosorbents. In the present study, the main effective binding sites have been identified by FT-IR in the spectral comparison of the native and atrazine interacted yeast biomass as biosorbent. FT-IR spectra display several vibrational bands indicating the complex nature of the native biosorbents viz. *P. kudriavzevii* and *C. laurentii* as shown in Figures 7 a,b and Figures 8 a,b respectively. The broad and strong bonds at 3446.79, 3336.85 $cm^{-1}$(*P. kudriavzevii* Atz-EN-01) and 3528.88 $cm^{-1}$ (*C. laurentii* Atz-EN-02) respectively, indicated bounded amide ($–NH_2$) groups. The peaks at 2935.66 $cm^{-1}$ (*P. kudriavzevii* Atz-EN-01), 2929.87 $cm^{-1}$ (*C. laurentii* Atz-EN-02) were attributed to the stretching vibrations of $–CH_2$ groups. The absorption bonds observed at 1658.78 $cm^{-1}$ for both *P. kudriavzevii* Atz-EN-01 and *C. laurentii* Atz-EN-02 attributed mainly C=O stretch broad band. The peak observed at 1402.25 $cm^{-1}$ for *P. kudiravzevii* Atz-EN-01 and 1427.32 $cm^{-1}$ for *C. laurentii* Atz-EN-02 were the medium stretching vibrations of OH in carboxylic acids.

FT-IR spectra of *P. kudriavzevii* Atz-EN-01 interacted with atrazine showed that the peaks expected at 3446.79, 3336.85, 1658.78 and 1402.25 $cm^{-1}$ had shifted, respectively 3519.68, 3402.25, 1669.46 and 1425.40 $cm^{-1}$ (Figure 7b). As for *C. laurentii* Atz-EN-02, the peaks expected at 3525.88, 1658.78 and 1427.32 $cm^{-1}$ had shifted to 3515.88, 1675.13 and 1425.40 $cm^{-1}$ (Figure 8b). The spectral analysis before and after atrazine binding clearly indicated that amide ($–NH_2$), carboxyl (–COOH) and OH in carboxyl groups are the predominant contributors in atrazine uptake for both the yeast strains.

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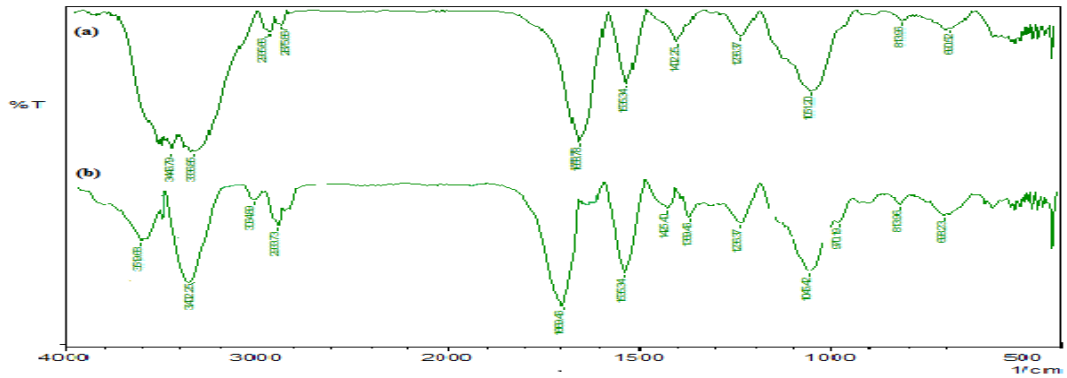


**Figure 7: FT-IR Spectrum of *P. kudriavzevii* Atz-EN-01 (a) before and (b) after Atrazine Adsorption.**

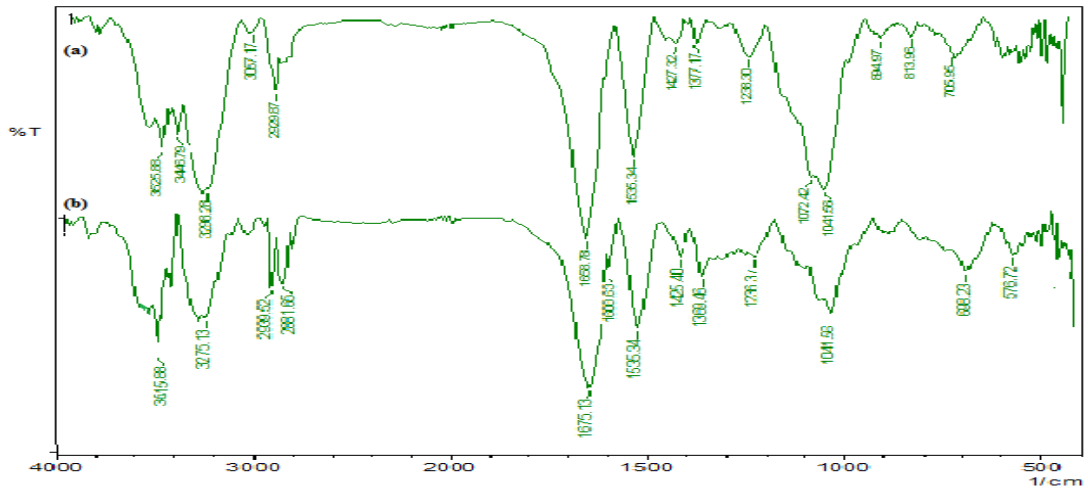


**Figure 8: FT-IR Spectrum of *C. laurentii* Atz-EN-02 (a) before and (b) after Atrazine Adsorption.**

## Scanning Electron Microscopy

Figure 9a and Figure 10a showed the smooth surface of the native biosorbents viz. *P. kudriavzeviii* Atz-EN-01 and C. *laurentii* Atz-EN-02*.* Adsorption of atrazine on yeast biosorbents were noted in Figure 9b and Figure 10b. Before adsorption, cells of both the adsorbents were visible as a whole. After adsorption, the cells of *P. kudriavzevii* Atz-EN-01 were not clumped and atrazine was adsorbed peripherally along the sides of the cell wall, whereas the cells of *C. laurentii* Atz-EN-02were less defined and more clumped, hence surface areas were reduced which led to less adsorption. The results of SEM analysis confirmed more amount of atrazine adsorption by *P. kudriavzevii* Atz-EN-01 compared to *C. laurentii* Atz-EN-02.

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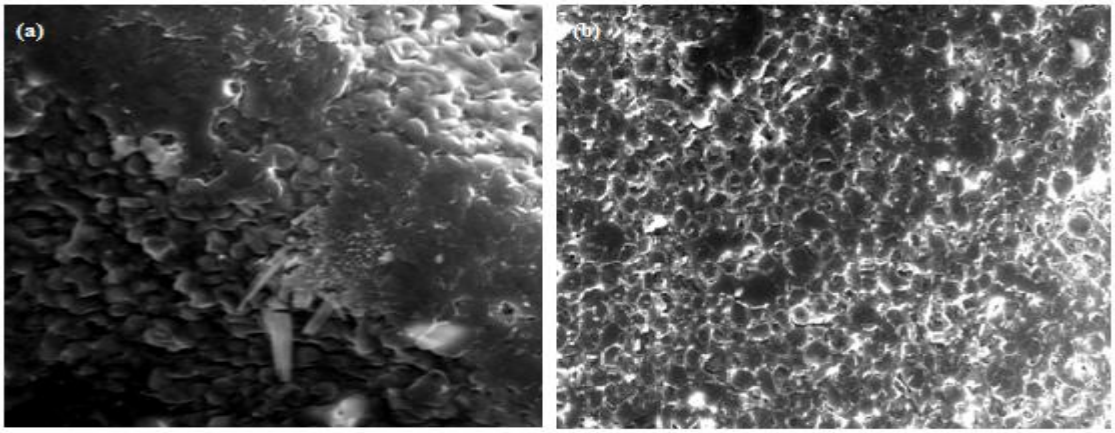


**Figure 9: Scanning Electron Microscopy Images of *P. kudriavzevii* Atz-EN-01 (a) before and (b) after Atrazine Adsorption.**

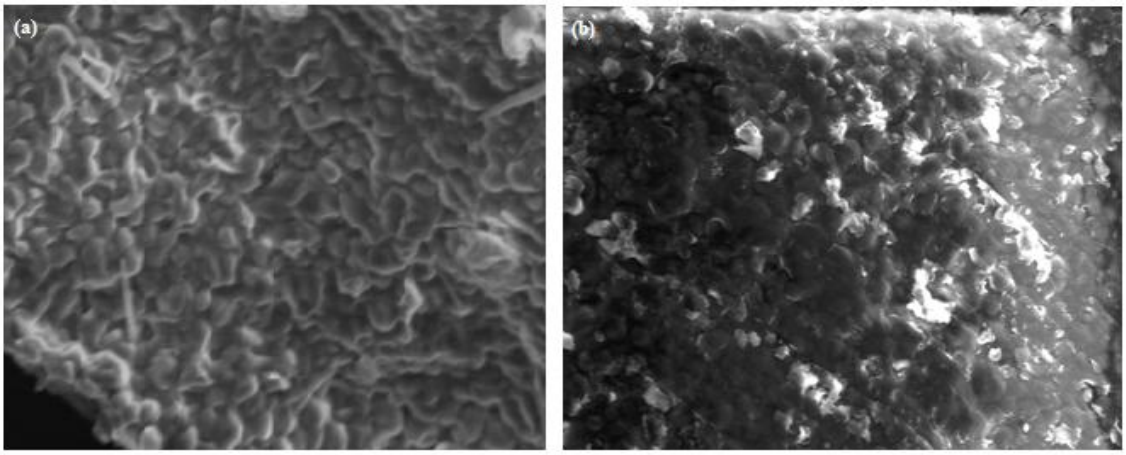


**Figure 10: Scanning Electron Microscopy Images of *C. laurentii* Atz-EN-02 (a) before and (b) after Atrazine Adsorption**.

## CONCLUSION

The potentiality of dead yeast biomass to adsorb atrazine from aqueous solution was demonstrated in this study. Equilibrium data were fitted to Langmuir, Freundlich and Temkin models, and the Langmuir model exhibited a best fit to the sorption data of atrazine confirming the homogeneous monolayer mode of adsorption. The biosorption of atrazine by both the yeast species followed pseudo-second order adsorption kinetics suggesting that adsorption process was controlled by chemisorption process. Thermodynamic analysis indicated that the process of atrazine adsorption was exothermic for both the yeast species. The plots of intraparticle diffusion model were not linear over the whole time range, depicting that more than one process was involved in the adsorption process. The most appropriate equation for defining the isotherm profile was Langmuir model showing the homogeneous mode of adsorption which was confirmed by SEM analysis. FT-IR analysis indicated that amide ($-NH_2$),

carboxyl (-COOH) were mainly involved in atrazine uptake by yeast species. Based on the results of the present study, it can be concluded that dead yeast *P. kudriavzevii* Atz-EN-01 could serve as a potential biosorbent for the efficient removal of atrazine from aqueous environment.

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