

## Uptake and Bioaccumulation of Copper by *Kluyveromyces Marxianus*

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### Abstract:

*The aim of the present study was to investigate the mechanism of Cu (II) bioaccumulation by live and immobilized cells of Kluyveromyces marxianus. The effects of operating parameters like pH, temperature and initial Cu (II) concentration on bioaccumulation were investigated. The concentration of the Cu (II) in the bioaccumulation media was varied between 20 to 500 mg/L. The optimum pH and temperature was found to be 5 and 35<sup>o</sup>C respectively for the growth and bioaccumulation of Cu (II). The mechanism of Cu (II) bioaccumulation involves both dependant and independent process of metabolism. Scanning Electron Microscopy (SEM) analysis were performed to study the surface binding and intracellular accumulation of Cu by the yeast. Clear morphological changes were observed in the SEM images of Cu (II) accumulated biomass. Presence of Cu in the yeast cells and functional groups involved in the bioaccumulation of Cu (II) by Kluyveromyces marxianus clearly indicated by Energy-dispersive X-ray spectroscopy (EDAX) analysis of the SEM and Fourier transform infrared spectra (FTIR) respectively. The changes in the FTIR indicated the chemical interaction between the functional groups and the metal ions. The protein analysis showed the decrease in the total protein due to the accumulation of copper ions in the metal binding proteins. The yeast cells are immobilized in sodium alginate and a column study was performed in order to find out the efficiency of the organism in maximum removal of heavy metal copper. In the column studies immobilized beads were able to remove copper ions at 50.54% with residual copper ions 138.96 mg/L from the solution with initial metal concentration of 300 mg/L with a flow rate of 10 ml/min and 15 cm bed height. The reusability of the immobilized beads was performed with desorption studies with 0.1M HCl. The beads showed no significant change till 3 cycles.*

**Keywords:** Copper, *Kluyveromyces marxianus*, SEM, FTIR, EDAX, Immobilized beads, Packed bed column, desorption

### INTRODUCTION

Day to day increase in industrialization and urbanization has contributed to the degradation of aquatic environment which in turn increased the biogeochemical cycling of toxic heavy metals causing the metal deposition into the aquatic and terrestrial ecosystems. Metals are not only important in industrial application but also play a significant role in applications such as medicine, electronics and nuclear power (Lloyd, 2002). Increase in metal processing and refining industries have resulted in the contamination of aquatic environment generating wastewater containing heavy metals (Dizadji et al, 2011). Copper, one among the heavy metal causing pollution, occurs as divalent or organic forms from the industry. Its concentration reaches 100 -120 mg/L from the waste generated from copper cleaning, copper plating industries which is much higher than that of permissible limit (1-1.5mg/L) (Chatterjee and Lalitagauri, 2008)

Conventional methods such as ion exchange, chemical oxidation, and chemical precipitation can be used for removing heavy metal ions from wastewater. However, this involves costly equipment and has high operational and energy requirements (Kujan et al, 2005). These methods utilize large amounts of chemicals, produce toxic sludge, and are inefficient at low concentrations of metals (<100 mg/L) (Donmez and Aksu, 1999). Biological materials are known for their potential to adsorb heavy metals. Microorganisms are potent bioremediators, removing metals via active or passive uptake mechanisms, commonly known as bioaccumulation and biosorption, respectively (Volesky, 2001). Bioaccumulation includes precipitation, intracellular accumulation and oxidation or reduction mechanisms. The passive biosorption occurs by physicochemical interaction between the cell surface and metal. It includes complexation with extracellular biological chelates/ligands and sorption onto cell surfaces (Batic and Raspor, 1998, Chojnacka, 2010). The main advantage of using growing

cultures in removal of heavy metals is to avoid the need for a separate biomass production (Wang et al, 2010, Yilmazer and Saracoglu, 2009). Heavy metals are harmful to microorganisms because of their strong affinity to form complex with the cell membrane constituents, causing loss of integrity and impairment of its function. But some microorganisms possessing metal resistance can overcome the harmful effects and could be utilized for biosorption of metals. The bacterial species used for metal biosorption act as potential biosorbent for heavy metal uptake (Regine and Volesky, 2000). Apart from bacteria, sea weeds and yeast were also used for heavy metal removal. The recent works shows the yeasts are good biosorbent for metal bioaccumulation (Sen and Ghosh, 2007; Udofia et al, 2009).

Yeast belongs to the group of microorganisms which are predominately used in modern biotechnology for bioremediation (Aksu and Donmez, 2000a). Optimization of their growth and metabolic activities requires ionic constituents such as inorganic and trace elements which are involved in metabolic and structural role. In high concentrations Cd (II), Cu (II) or Zn (II) and other metal ions can be toxic while in low concentrations they stimulate growth enzyme activity (Batic and Raspor, 1998). The metal uptake by the organism is mainly depending on the metal binding proteins (Bishnoi and Garima, 2005). Thus the aim of the present study was to optimize growth parameters and to determine the sorption mechanism for copper uptake by the potent yeast, *Kluyveromyces marxianus* and to find the maximum ability of the immobilized cells in removing Cu (II) ions from aqueous solution.

## **MATERIALS AND METHODS**

### **Microorganism and Media**

*Kluyveromyces marxianus* (MTCC 95) yeast was obtained from the Institute of Microbial Technology, Chandigarh, India and cultivated at 25 °C in a liquid medium at pH 6. The strain was maintained on YEPD Agar. The general media for growth of yeast, *Kluyveromyces marxianus* consisting of (g/L) Malt Extract 3, Yeast extract 3, Dextrose 10 and Peptone 5 in distilled water. The pH of the medium was maintained at 7 using 0.1N HCl. Medium composition have a remarkable effect of bioaccumulation because of its cell affinity towards the metal bindings (Sersy and Sharouny, 2007). The optimized media used for the present study contained in g/L Yeast extract 2, Lactose 3 and Peptone 2.5 containing different concentration of copper in distilled water. All media were autoclaved at 15 kPa and 120 °C for 20 min.

### **Stock solution preparation**

The bioaccumulation capacity of living *Kluyveromyces marxianus* was investigated with Cu (II) stock solution prepared by dissolving 3.928 g of CuSO<sub>4</sub> in 1000 mL deionized water, shaking it at 150 rpm for 15 min to get complete dissolution. The bioaccumulation medium was prepared by diluting this solution to required concentration in the growth media.

### **Determination of heavy metal-resistant by plate diffusion method**

Heavy metal resistant was determined by plate diffusion method. The metal solution was used in varying concentrations ranging from 20 to 500 mg/L. Stock solution of the metal salt was prepared in sterile distilled water and was added to the agar medium (pH-7.0) in various concentrations which were then inoculated with yeast. The plates were incubated at 25<sup>0</sup>C for 48h. The lowest concentration of the metal, which inhibits the growth of the microorganism, was considered as the Minimal Inhibitory Concentration (MIC) of the metal against the yeast (Rani et al, 2010, Singh et al, 2010)

### **Batch study for optimization**

Batch adsorption experiments were carried out by shaking the flasks at 150rpm for a period of contact time using a rotary shaker. For the growth and metal analysis samples were centrifuged and the concentration of metals in the supernatant solution was measured using AAS. The effect of pH on the metal removal capacity of the yeast was investigated. The growth medium was prepared and its pH was adjusted for a pH range of 3 to 7. Due to hydrolysis and deposition of copper ions at higher pH above 7, experiments could not be performed (Wang et al, 2010). Further, temperature effect in the range of 15<sup>0</sup>C to 45<sup>0</sup>C and initial metal concentration of

varying range of 20 to 500 mg/L were also been studied. For all the optimum studies the medium was inoculated with 1% v/v of inoculum and samples were analyzed.

### **Model for growth of yeast with copper**

Growth medium containing a solution of 20-100mg/L Cu (II) ions was prepared and its pH was adjusted to optimum value. The medium was then inoculated with the inoculum and incubated at optimum temperature. The growth kinetics of the organism was determined for varying initial metal concentration of optimized condition and the Monod curve was plotted (Aksu and Donmez, 2000b).

The relationship of specific growth rate to substrate concentration often assumes the form of saturation kinetics. In this case we assumed initial copper concentration (Substrate), as growth rate limiting substrate for the microorganism. The Monod model is stated as:

$$\mu = \frac{\mu_{max} * [S]}{K_s + [S]}$$

Where,

$\mu$  = Specific growth rate, time<sup>-1</sup>

$\mu_{max}$  = Maximum specific growth rate when  $S \gg K_s$

[S] = Substrate concentration, mg/L

$K_s$  = Saturation constant or half-velocity constant, mg/L

### **Estimation of total protein content of the yeast**

Growth medium with a solution of 20-500 mg/L Cu (II) was prepared and batch study was conducted. At the stationary phase of the organism, 10ml was withdrawn and centrifuged at 10,000rpm for 5minutes. The supernatant was discarded and the biomass was dried. Dried samples of washed cells were incubated in 5ml of 1N NaOH at 90°C for 10minutes to solubilize cellular protein. The cellular protein was estimated using Lowry method (Hassen *et al.*, 1998).

### **Immobilization of the yeast**

In the stationary phase of growth, yeast cells were centrifuged and the biomass was resuspended in 3% sodium alginate. The ratio of Na-alginate to biomass was 100:3. This mixture was dropped into 0.5 M calcium chloride solution. The drops of Na-alginate solution gelled into spheres upon contact with calcium chloride solution. Ca-alginate immobilized yeast particles were stored in calcium chloride solution at 4°C for 24 h to complete gel formation. In this way insoluble and stable immobilized beads were obtained (Banerjee *et al.*, 2007 and Tsekova *et al.* 2010) and used for further immobilization studies. The viability of the cells is tested before and after sorption of metal.

### **Column studies**

Continuous flow experiments were conducted in a glass column (Inner diameter=2cm, Total column height=35cm). At the bottom of the column, sterilized non-absorbent cotton was placed followed by 1cm high layer of glass beads. The column was then packed densely with immobilized beads and operated in an up flow mode at room temperature. The schematic diagram of the column study is shown in fig: 1. The flow rate was regulated with peristaltic pump. The column study was done at the flow rate of 10ml/min the packing height is varied (5cm, 10 cm and 15cm) in order to find out the efficiency of the organism in removal of copper metal ions.

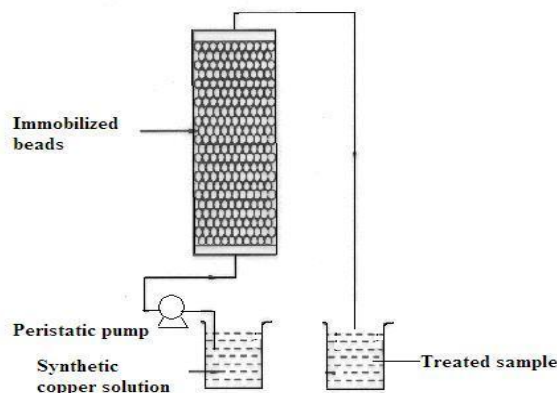


Fig 1: Schematic diagram of packed bed column

### Desorption study

For desorption, the immobilized beads containing adsorbed Cu metal ions, was packed through the column and desorbing solution was passed at a flow rate of 2 ml/ min for 4 h. The capacity of copper desorbed is more in the 0.1 M HCl solutions which is used for desorption. The desorbed solution was analyzed in atomic absorption spectrophotometer

### Analytical methods

Microbial growth was monitored by measuring the optical density of the cultural medium every two hours at 600 nm using a spectrophotometer. Determinations of copper ions uptake by *Kluyveromyces marxianus* were done using an atomic absorption spectrophotometer (AAS).

## RESULTS AND DISCUSSION

### Cu<sup>2+</sup> ions sensitivity

MIC was performed in order to evaluate the level of resistance of the organism to the metal Cu (II) ions. MIC was noted when the isolates failed to grow on plates. The minimum concentration of metal in the medium inhibiting complete growth was taken as the MIC (Zaki and Farag, 2010). The yeast cells were able to grow in the growth medium containing the metal concentration of up to 600ppm. Above 600ppm no growth was observed in the medium agar. *E. coli* strain carrying the plasmid-borne Cu resistance genes (pco) could tolerate approximately 5-fold higher concentrations of cupric ions than sensitive strain (Brown et al. 1992). *Kluyveromyces marxianus* strain was also able to tolerate 5 folds higher concentration of Cu (II) ions.

### Effect of pH

The effect of pH has been reported as a key parameter in most biological processes which controls the growth as well as the metal adsorption capacity. The cell surface metal binding sites and availability of metal in solution are affected by pH. At low pH, the cell surface sites are closely linked to the H<sup>+</sup> ions, thereby making these unavailable for other cations. However, with an increase in pH, there is an increase in ligand with negative charges which results in increased binding of cations (Ahuja et al, 1999). The increase of pH resulted in an increased negative charge on the surface of the cell which favored electrochemical attraction and adsorption of metal (Gourdon et al 1990). The effect of pH in the presence of copper is shown in the Fig 2. The percentage uptake of copper ion by the yeast species ranged from 47.8-75.1%. Metal uptake by the living biomass was low initially at pH 3 before rising to a maximum value of pH 5 and then dropped at pH 7. The highest uptake of copper 75.1 mg/L was obtained at pH 5 for *Kluyveromyces marxianus*. The optimum pH for *Rhizopus arrhizus* for removal of Cu (II) was found to be 4.5(Dursun et al,2003)The increase in copper uptake at range 3 to 5 is

due to the strong relations of bioaccumulation to the number of surface negative charge, which depends on the dissociation of functional group. The adsorption of copper ions is mainly due to the ionic attraction. At low pH the cell surface becomes positive and reduces the sorption capacity of metal and the biomass (Bishnoi et al, 2004, Bunluesin et al 2007, Karin et al, 2003.)

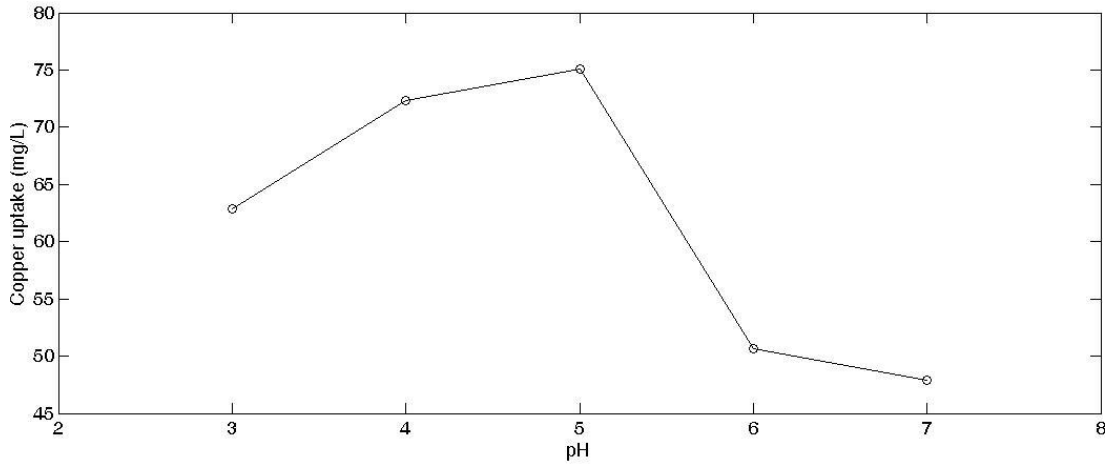


Fig 2: The effect of pH on copper uptake by *Kluyveromyces marxianus*( Initial metal concentration =20mg/L, Temperature =30<sup>0</sup>C)

### Effect of Temperature

The effect of temperature on the copper uptake by the yeast is shown in the Fig 3. The yeast exhibited optimum growth at 35<sup>0</sup>C with maximum uptake of 70.22 mg/L. When the growth temperature is reduced, the initial lag phase extends, the growth rate decreases and the final cell number usually decreases. At low temperatures, the organism produced longer lag phase extending upto 40h. An optimum temperature of 30<sup>0</sup>C for uranium uptake by *Kluyveromyces marxianus* was observed (Bustard et al, 1997).The maximum removal of copper occurred at 35<sup>0</sup>C.

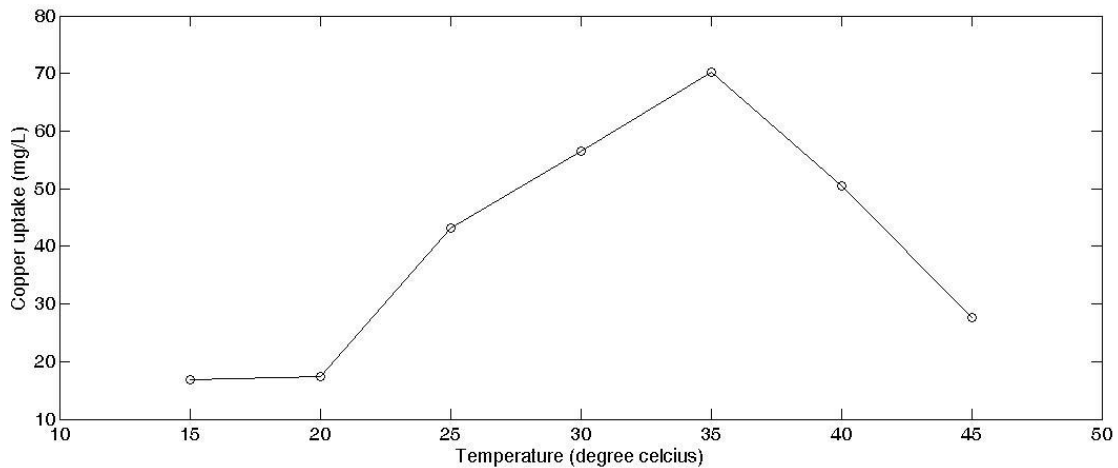


Fig 3: The effect of Temperature on copper uptake by *Kluyveromyces marxianus* (Initial metal concentration =20mg/L, pH =5)

### Effect of Initial Metal Concentration

The effect of initial copper concentration on the growth of the yeast was upto 300 mg/L of copper which is shown in Fig 4. Above the 300 mg/L *K. marxianus* growth was sensitive to high concentration copper. The % removal of copper ions varied from 95.99 % - 3.32%. The maximum % removal of the yeast was seen at

20mg/L of copper concentration indicates that the lower copper concentrations favored higher % removal. At higher concentration the toxic effects of copper metal on cell growth causes the reduction in removal which is due to the water and osmotic relations disturbance of the cells such as decreased cell membrane stability or changed ions relations of the cells (Georgieva, 2008). The tolerance of the yeast towards the high concentration of the copper metal may be due to the transport of metal to the mitochondria where it helps in respiration of the cells (Ting and Choong, 2009)

The residual biomass from the yeast strain *Kluyveromyces marxianus* IMB3 had an observed maximum biosorption metal capacity of 120mg/g dry weight of biomass (Anagnostopoulos et al, 2010). The biosorption of cadmium and lead ions from artificial aqueous solutions using waste baker’s yeast biomass showed the highest metal uptake of 17.49mg/g for Pb was obtained by ethanol treated yeast cells (Goksungur et al, 2005). The maximum uptake capacity of the baker’s yeast was 20.4mg/g dry biomass when using 150mg/L initial lead (II) concentration (Skountzou et al, 2003). The residual biomass for the *Kluyveromyces marxianus* yeast strain in present study showed a maximum biosorption capacity of 95.99 % of Cu (II) ions.

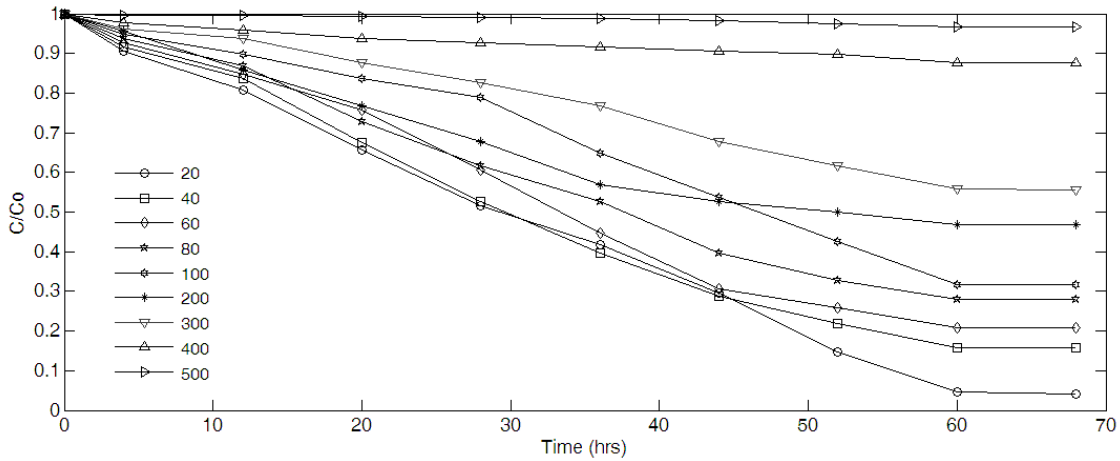


Fig 4: The Copper uptake by yeast on different initial metal concentration

**Monod curve**

The cell growth increased with increase in initial concentration of copper. The specific growth rate as a function of initial substrate concentration is presented in Fig 5. It was inferred that till the initial substrate concentration of 40mg L<sup>-1</sup>, the specific growth rate increases. After 60mg L<sup>-1</sup> of substrate concentration, the specific growth rate is found to be stationary upto 200mg L<sup>-1</sup> of copper.

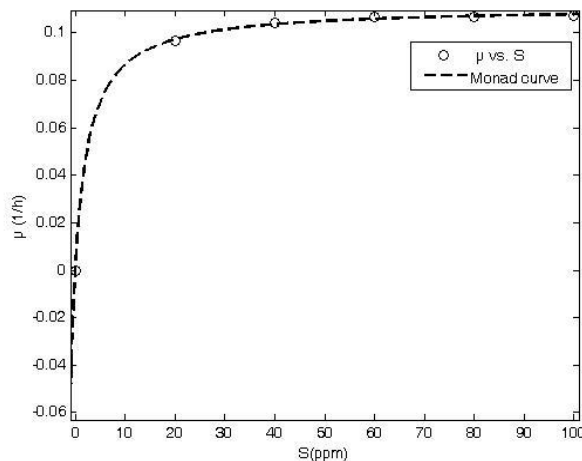


Fig 5: Monod curve for the growth of yeast

From the regression equations we are able to determine the growth kinetic parameters ( $K_s$  and the  $\mu_{max}$ ). The half saturation constant ( $K_s$ ) is 2.806 mg/L, and the maximum specific growth rate ( $\mu_{max}$ ) was  $0.1108\text{ h}^{-1}$ .

**Estimation of protein concentration**

To determine if enhanced accumulation of the metal inside the cell may be related to the synthesis of proteins, attempts were made to estimate the proteins from culture of yeast grown in the presence of different concentration of copper. As the concentration increases the total protein concentration decreases showed the copper concentration affects the protein content in the cells. The initial concentration of copper till 80ppm does not showed any significant changes in protein content which is showed in Fig 6.

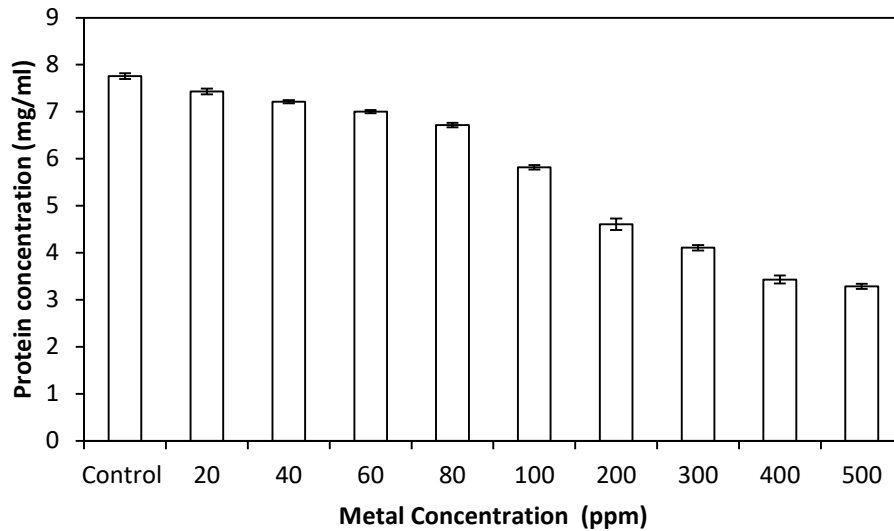


Fig 6: The effect of initial metal concentration on total protein.

**Intracellular accumulation of Cu**

When extracellular concentration of the metal ion is higher than the intracellular metal concentration the metal ions can penetrate into the cells through the cell wall by free diffusion (Skountzou et al, 2003). SEM with EDAX was used as a tool to study the mechanism of the metal inside the cytoplasm. It can be seen from the Fig 8a the Cu accumulated biomass exhibited dense granule. These dense granules were not observed in the Cu free control Fig 7a. Further the EDAX confirmed that the dense granules are composed of Cu, Which can be seen in Fig 7b and 8b. The adsorption outside the cell wall is due to the binding of copper ions to the cell wall components (Sun and Shao, 2007).

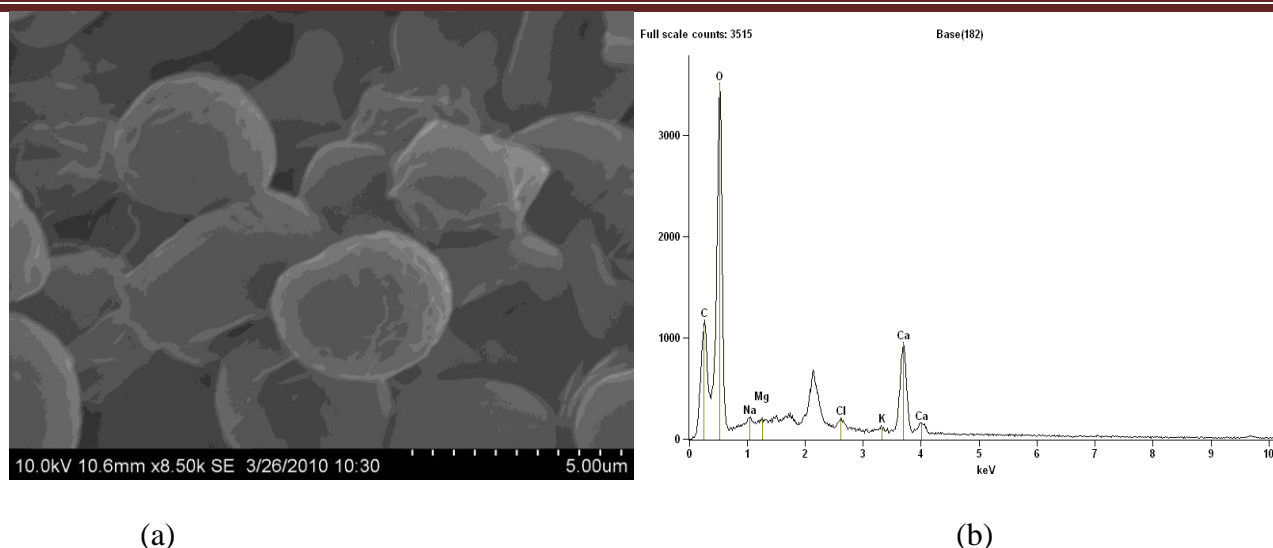


Fig 7: SEM & EDAX Micrograph of *K. marxianus* before copper accumulation

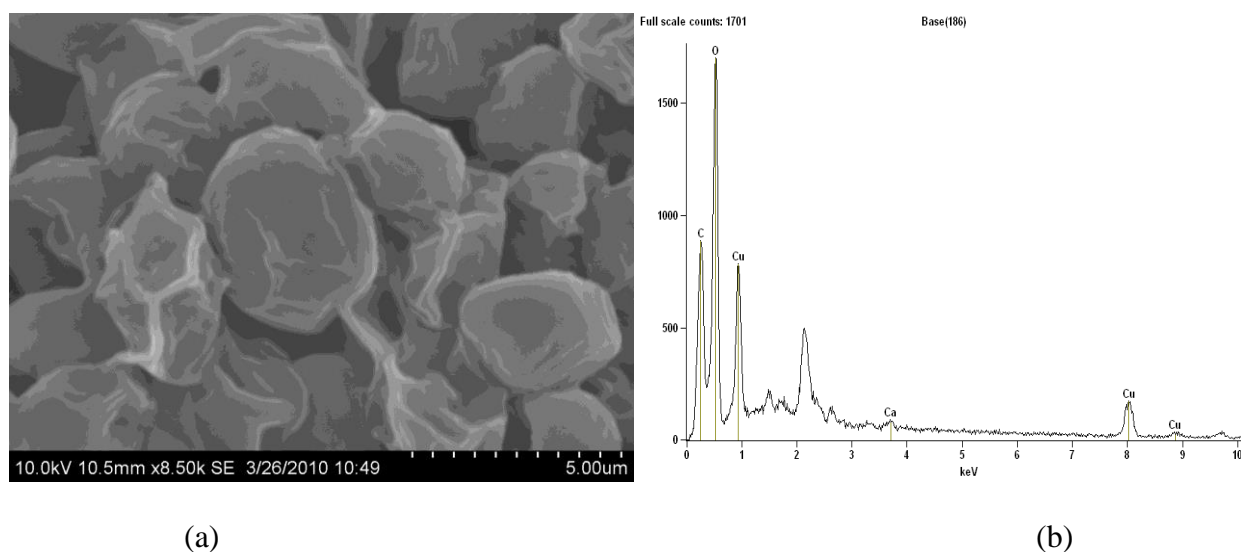


Fig 8: SEM & EDAX Micrograph of *K. marxianus* after copper accumulation

### FTIR study

FTIR is used to identify the types of chemical bonding in a molecule and to find the ability of the spectrum in wide range of compounds (Santos et al, 2010). The copper uptake capacity of the yeast was studied in order to understand the mechanism of Cu accumulation. Earlier, it was observed that pH 5 was found to be the optimum for the growth of the yeast and 20 mg L<sup>-1</sup> concentration of Cu never inhibited the growth. The Fourier transform IR spectra were made in this study for both metal free and metal accumulated yeast and given in Fig 9(a) and (b).

The FTIR spectra confirmed the presence of functional groups responsible for bioaccumulation (amino, carboxyl and nitrile groups). The metal accumulated in yeast biomass showed variation in peak values in comparison with the metal free yeast biomass. Intense peaks which are the characteristic of Alkyne are observed at the frequency in the range of 2300-2500 cm<sup>-1</sup>. A narrow shift in the peak values from 3600- 3974 cm<sup>-1</sup> characteristic peaks for primary amines and amides were observed in the metal accumulated yeast biomass. This may be due to the OH, NH and acetamide groups of the yeast cell wall. A distinct peak at the 1742 cm<sup>-1</sup> of the metal accumulated biomass can be attributed to the C-O stretching band of the aminoacids. The yeast



biomass shows strong amide I and amide II absorption bands at 1647 and 1550  $\text{cm}^{-1}$  which was the characteristic for protein molecules (Karin et al, 2003). The FTIR spectra clearly indicated the involvement of amines and amides are responsible for the bioaccumulation.

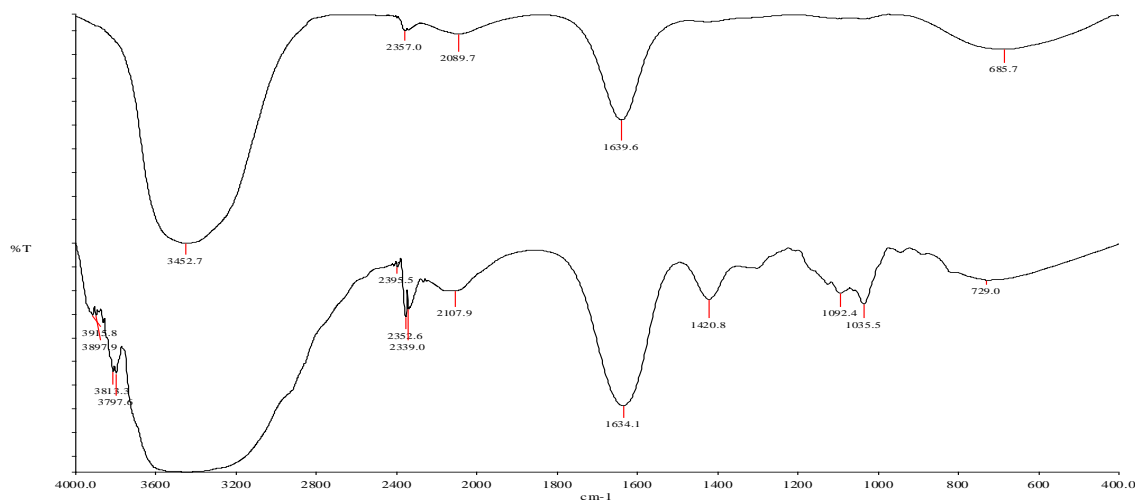


Fig 9: The FTIR spectra for both metal free (a) and metal accumulated (b) yeast.

#### Metal uptake of immobilized beads using column study

For industrial requirement the sorbent should be utilized as a fixed or expanded bed to avoid much pressure drop across the bed. The free cells are not suitable for column packing in industrial application due to their low mechanical strength and small particle size and excessive hydrostatic pressures are required to generate suitable flow rates. High pressures can cause disintegration of free biomass (Saifuddin and Raziah 2007). Immobilized biomass have many advantages including better reusability, high biomass loading and minimal clogging in continuous flow systems. Cells are immobilized in a support matrix to enhance the stability and ease of use of whole cell systems. In this study immobilization of yeast in an alginate matrix was performed, this matrix is an inexpensive and biodegradable polymer, ability to maintain high stability of the cell for extended period of time and reusability. Using this matrix, column experiments were conducted to study the effects of metal binding by the yeast biomass under flow conditions.

Fig.10 represents the breakthrough curve for copper (II) passed through the column. The curve shows the amount of metal remaining after solutions at pH 5 were passed through the column. The copper concentration of 300 mg/L solution was continuously passed to the column which was packed immobilized alginate beads in order to compare the efficiency of the beads with the free cells. The cells remain viable after the sorption showed the metal concentration does not affect the viability of the cells. Thus finally the immobilized beads were able to treat 1500ml of the influent solution and discharge it as metal free solution. The percentage removal of free and the alginate immobilized beads were given in Table 1. From the table we can reveal that alginate beads are efficient in removal of Cu (II) from contaminated industrial effluent containing 300 mg/L of Cu (II) metal ions. Usually the waste discharged from industries contains 100-120 mg/L Cu (II) ions which are less than the concentration used in the present study.

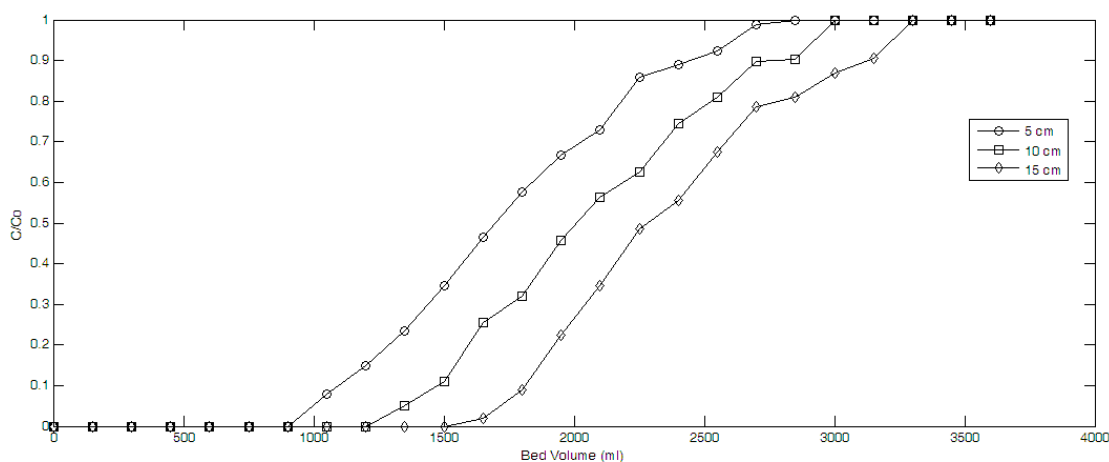


Fig 10: Effect of bed height on uptake of copper by immobilized *K. marxianus* (Co=300ppm, pH=5, Temperature=30°C, Flow rate=10 ml, Bed height = 5, 10 and 15cm)

Table 1 Effect of contact time on accumulation of copper by free and immobilized biomass of *K.marxianus*

Time (hrs)	Percentage of bioaccumulation at 300mg/L of copper metal concentration		
	Blank alginate beads	Alginate beads with <i>K.marxianus</i>	Free biomass of <i>K.marxianus</i>
6	2.75	3.43	3.76
12	4.34	10.3	6.32
24	5.20	20.34	17.3
48	5.24	37.33	38.31
72	5.24	50.44	44.52

### Reuse of Immobilized beads

The stability and potential recyclability of the biomass were assessed by monitoring the changes in recoveries through adsorption desorption cycles. It was seen that the immobilized biomass in the matrix has good stability for extended period of time. The 0.1 M HCl was more effective in desorption of copper ions. The ability to desorb and recover bound metals and regenerability of the biomass sorbent are the key factors for improving the economy of the process (Subhashree and Rai 2001). With the advantages of high metal biosorption capacity, satisfactory recovery efficiencies and persistence on repeated use. There was no significant decrease in adsorption till 3 cycles after that the desorption capacity of the immobilized was decreased are shown in fig 11.

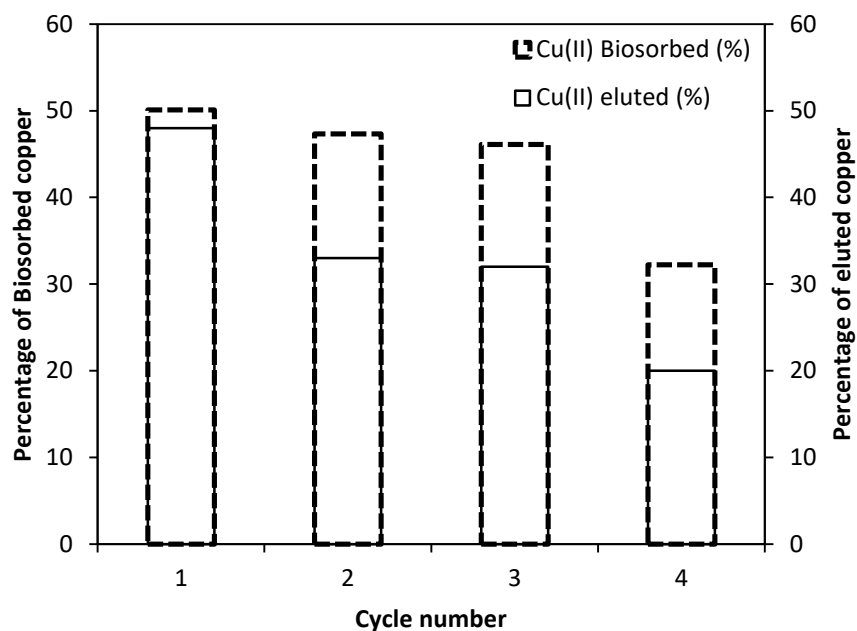


Fig 11. Biosorption-desorption of Cu (II) by immobilized beads in four consecutive cycles.

## CONCLUSION

The uptake of Copper by live *Kluyvernomycetes marxianus* is a two-step process. The Cu (II) ions first bind on to the surface of the cell wall by charge based interaction with the functional groups. The second step involves the intracellular accumulation by metabolism dependant process. The FTIR study reveals that the amide and amine groups are involved in the surface binding of the Cu (II) ions with the cell wall. The SEM and EDAX confirm the surface binding of Cu ions on to cell wall. The present study is an initiative to study mechanism of the yeast interaction with metals.

This study was done to evaluate the removal of Cu (II) ions continuous process from solution through immobilized *Kluyvernomycetes marxianus* in packed bed column. Before using the biomass for large scale metal removal and recovery, it will be necessary to optimize the bed height for maximum removal of metal in laboratory conditions. Further, the ability of the immobilized beads to desorption and recover bound metals and regenerability of the beads are the key factors for improving the economy of the process. With the advantages of high metal uptake capacity, satisfactory recovery efficiencies and persistence on repeated use, biomass of *Kluyvernomycetes marxianus* appears to hold great potential for the removal and recovery of heavy metals from polluted waters.

## REFERENCES

- i. Ahuja, P, Gupta R. and Saxena R.K,(1999). Sorption and desorption of cobalt by *Oscillatoria anguistissima*. *Current Microbiology*, 39: 49-52 (4 pages).
- ii. Aksu .Z and Donmez .G (2000 a). Combined effects of sucrose and copper (II) ions on the growth and copper (II) bioaccumulation properties of *Candida sp.* *Journal of chemical technology and biotechnology*, 75(9), 847-853(7 pages).
- iii. Aksu.Z and Donmez. G. (2000 b). The use of molasses in copper (II) containing wastewaters: effects on growth and copper (II) bioaccumulation properties of *Kluyveromyces marxianus*. *Process Biochemistry*, 36 (5), 451-458. (8 pages).
- iv. Anagnostopoulos. V.A, Bekatorou .A and Symeopoulos. B.D (2010). Contribution to interpretation of metal uptake dependence upon the growth phase of microorganisms. *Journal of Radio analytical and Nuclear Chemistry*, 287 (2), 665-671(7 pages).

- v. *Batic.M and Raspor.P (1998). Uptake and bioaccumulation of Cr (III) yeast Saccharomyces cerevisiae. Food technology and biotechnology, 36(4), 291-297 (7 pages).*
- vi. *Bishnoi N.R and Garima (2005). Fungus –An alternative for bioremediation of heavy metals containing waste water – a review. 64(2), 93-100 (8 pages).*
- vii. *Bishnoi N.R, Pant.A and Garima (2004). Biosorption of copper from aqueous solution using algal biomass.63 (4), 813-816(4 pages).*
- viii. *Brown, N.L., Rouch, D.A. & Lee, B.T.O. (1992). Copper resistance determinants in bacteria. Plasmid 27, 41-51(11 pages).*
- ix. *Bunluesin.S, Pokethitiyook.K.P, Upatham.S, Lanza G.R. Batch and continuous packed column studies of Cadmium biosorption by Hydrilla verticillata biomass. Journal of bioscience and bioengineering, 103(6), 509-513 (5 pages).*
- x. *Bustard.M, Donnellan. N, Rollan. A and McHale A.P (1997). Studies on the biosorption of uranium by a thermotolerant, ethanol-producing strain of Kluyveromyces marxianus. Bioprocess Engineering, 17(1), pp 45-50 (6 pages).*
- xi. *Chatterjee .A and Lalitagauri. R (2008), Biosorption of Cu (II) by immobilized biomass of Bacillus cereus M<sup>1</sup><sub>16</sub> from aqueous solution.67, 629-634 (6 pages).*
- xii. *Chojnacka.K (2010), Biosorption and bioaccumulation – the prospects for practical applications, Environment International 36, 299–307 (9 pages).*
- xiii. *Dizadj.N, Abootalebi Anaraki.N, Nouri.N(2011), Adsorption of chromium and copper in aqueous solutions using tea residual, Int. J. Environ. Sci. Tech, 8(3),631-638( 8 pages).*
- xiv. *Donmez.G, Aksu.Z (1999). The effect of copper (II) ions on the growth and bioaccumulation properties of some yeast. Process Biochem, 35(1-2), 135-142(8 pages).*
- xv. *Dursun A.Y., Uslu. G., Tepe O., Cuc i .Y., Ekiz H.I.(2003) A comparative investigation on the bioaccumulation of heavy metal ions by growing Rhizopus arrhizus and Aspergillus niger, Biochemical Engineering Journal 15(2), 87–92,(6 pages)*
- xvi. *Georgieva, N., 2008. Growth of Trichosporon cutaneum R 57 in the presence of toxic concentration of cadmium and copper. Int. J. Agri.Biol10, 325-328(4 pages)*
- xvii. *Goksungur. Y., Uren S. and Guvenc U. (2005) Biosorption of cadmium and lead ions by ethanol treated waste baker's yeast biomass. Bioresource Technology, 96 (1), 103-109 (7 pages).*
- xviii. *Gourdon, R, Bhande S., Rus E. and Sofer S.S.(1990). Comparison of Cadmium biosorption by gram positive and gram negative bacteria from activated sludge. Biotechnology Letters, 12: 839-842 (4 pages).*
- xix. *Hassen A, Saidi N, Cherifh M and Boudabous A (1998). Effects Of Heavy Metals On Pseudomonas Aeruginosa and Bacillus Thuringiensis , Bioresource Technology, 65, 73-82 (10 pages).*
- xx. *Karin P.K, Cecal.A, Drochioiu.G, Pui.A, Humelnicu.D (2003), Saccharomyces cerevisiae as uranium bioaccumulating material: the influence of contact time, pH and anion nature. Nukleonika 48(3), 121–125, (5 pages).*
- xxi. *Kujan .P, Prell.A, Safar.H, Sobotka.M, Rezanka.T, Holler.P.(2005). Removal of copper ions from dilute solutions by Streotomyces roursei mycelium .Comparison with yeast biomass, Folia microbiology, 50(4) 309-313, (5 pages).*
- xxii. *Lloyd. JR. (2002). Bioremediation of metals; the application of microorganisms that make and break minerals. Microbiology Today, 29, 67-69 (3 pages).*
- xxiii. *Rani M.J, Hemambika .B, Hemapriya. J and Kannan V.R (2010).Comparative assessment of heavy metal removal by immobilized and dead bacterial cells a biosorption approach. Journal Environmental Science &Technology, 4(2), 77-83 (7 pages).*
- xxiv. *Regine HSF, Volesky B (2000). Biosorption: a solution to pollution. Int. Microbiol. 3: 17-24 (7pages).*
- xxv. *Saifuddin, N. and Raziah, A.Z. (2007).Removal of Heavy Metals from Industrial Effluent Using Saccharomyces Cerevisiae(Baker's Yeast) Immobilised in Chitosan/lignosulphonate Matrix, J. Appl. Sci. Res, 3(12), 2091-2099(9 pages).*

- xxvi. Santos.C, Marcelo E. F., Kozakiewicz.Z, Lima.N(2010), *Fourier transform infrared as a powerful technique for the identification and characterization of filamentous fungi and yeasts*, *Research in Microbiology* 161(2): 168-175,(8 pages)
- xxvii. Sen M, Ghosh DM (2007). *Biosorption of Cr (VI) by resting cells of Aspergillus sp.* *Iranian J. Environ. Health Sci. Eng.* 4(1): 9-12(4 pages)
- xxviii. Sersy.N.A, Sharouny.EE, (2007) *Nickel biosorption by free and immobilized cells of marine Bacillus subtilis N10*, *Biotechnology*, 6(3), 316-321 (6 pages).
- xxix. Singh .V, Chauhan.P.K, Kanta.R, Dhewa .T, Kumar.V. (2010). *Isolation and characterization of Pseudomonas resistant to heavy metals contaminants.* *International Journal of Pharmaceutical Sciences Review and Research* 3(2) 164-167 (4 pages).
- xxx. Skountzou .P, Soupioni. M, Bekatorou. A, Kanellaki. M, Koutinas. A.A, Marchant. R and Banat .I.M.(2003).*Lead(II) uptake during baker's yeast production by aerobic fermentation of molasses.* *Process Biochem*, 38 (10), pp 1479-1482 (4 pages).
- xxxi. Subhashree Pradhan and L.C. Rai (2001).*Copper removal by immobilized Microcystis aeruginosa in continuous flow columns at different bed heights: study of the adsorption/desorption cycle*, *World J Microb Biot*, 17, 829–832 (4 pages).
- xxxii. Sun.F and Shao.Z (2007). *Biosorption and bioaccumulation of lead by Penicillium sp. Psf-2 isolated from the deep sea sediment of the Pacific Ocean.* *Extremophiles* 11(6), 853–858 (6 pages).
- xxxiii. Ting. A.S.Y, Choong C.C.(2009). *Bioaccumulation and biosorption efficacy of Trichoderma isolate SP2F1 in removing copper (Cu (II)) from aqueous solutions.* *World J Microbiol Biotechnol.* 25(8), 1431– 1437(7 pages).
- xxxiv. Udofia.G.E, Essien. J.P., Eduok.S.I and Akpan.B.K(2009) *Bioaccumulation of heavy metals by yeasts from Qualboe estuary mangrove sediment ecosystem, Nigeria*, *Afr J Microbiol Res*, 3(12) 862-869 (8 pages).
- xxxv. Volesky. B (2001). *Detoxification of metal bearing effluents: biosorption for the next century.* *Hydrometallurgy*, 59(2-3), 203-216 (14 pages).
- xxxvi. Wang.J, Hua.X, Liu.Y, Xie.S and Bao.Z (2010). *Biosorption of uranium (VI) by immobilized Aspergillus fumigatus beads.* *J Environ Radioactiv*, 101 (6), 504-508 (8 pages).
- xxxvii. Yilmazer.P and Saracoglu.N.(2009) *Bioaccumulation and biosorption of copper(II) and chromium(III) from aqueous solutions by Pichia stipitis yeast.* *J Chem Technol Biotechnol*; 84: 604–610 (7 pages).
- xxxviii. Zaki .S, Farag.S.(2010), *Isolation and some molecular characterization of some copper biosorped strain.* *Int. J. Environ. Sci. Tech*, 7(3), 553-560 (8 pages).