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Biosorption of Copper Ion using immobilized *Saccharomyces Cerevisiae* in a continuous Packed-Bed column

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Abstract: This study was investigated the removal of copper (II) from aqueous solution using *Saccharomyces cerevisiae* in a packed bed. *Saccharomyces cerevisiae* was immobilized to enhance the stability and ease of treatment in continuous systems. The experiment was conducted to study the effect of important parameters such as effect of initial concentration, bed height and flow rate. The results indicated that the efficiency of copper removal increased with increasing of bed height at the lowest flow rate. Whereby, breakthrough time decreased with increasing of initial concentration of copper (II).

Key words: Biosorption; Immobilized *Saccharomyces Cerevisiae*; Continuous packed-bed column; Breakthrough curve

1. Introduction

Nowadays, biosorption becomes one of the great alternative methods to remove heavy metal from aqueous solution due to the several advantages such as low cost, high efficiency in removal heavy metal, minimization of chemical used and regeneration of biosorbent (Areco and Afonso, 2010; Saifuddin and Raziah, 2007). Biosorption can be expressed as the uptake of heavy metal by using biological material as adsorbents, called biosorbents derived from by product or waste materials and microorganisms (Jonglertjunya, 2008). Different types of biosorbent that have been used to remove heavy metals from aqueous solution are mostly prepared from agricultural wastes (tobacco dust (Qi and Aldrich, 2007), black gram husk (Ahmad et al., 2009) and peanut shell (Krowiak et al., 2011)) and biologically inactive microorganisms (bacteria (Colak et al., 2011), algae (Flouty and Estephane, 2012) and fungi (Pang et al., 2011)).

Of late, there is an increasing interest in using yeast, a single-celled fungus, as biosorbent to remove heavy metals from aqueous solution since it is easy to grow, produces high yield of biomass and can be manipulated morphologically and genetically (Fu and Wang 2011). Among all the yeasts, *Saccharomyces cerevisae* (*S.cerevisiae*) is one of the popular ones that have been used extensively as biosorbent for heavy metal removal from aqueous solution by numerous researchers. This is on account of its salient features such as ease of cultivation at large scale with cheap media, available in abundance as a waste product of the fermentation industry and ease of manipulation at molecular level to study the metal-microbe interactions (Wang and Chen, 2006). Various forms of *S. cerevisae* have been used as biosorbents to remove heavy metals from aqueous solution, for instance treated and untreated cells (Goksungur et al., 2005), live and dead flocculent cells (Machado et al., 2009), engineered cell (Kotrba and Ruml, 2010), as well as immobilized and freely suspended cells (Kumar and Rao, 2011). Of late, immobilized cells has drawn considerable research attention because it offers several advantages over the freely suspended cells such as enhanced stability, reusability and mechanical strength, ease of treatment and minimal clogging in continuous systems (Peng et al., 2010). For instance, Li et al. (2008) discovered that the thermal stability and reusability of immobilized *S. cerevisiae* cells were better than the freely suspended cells in which the immobilized cells managed to retain their activity at higher temperature and they could be reused up to 7 times. Moreover, Martins et al. (2013) claimed that the mechanical strength of immobilized cells is higher than the freely suspended cells since immobilization prevents the cells from diffusing to surroundings.

Copper ion is one of the hazardous heavy metal to the human health and environment (Khan et al., 2013). Usually copper ions are widely used in metal finishing, plating, dyeing and petroleum industries (Karthika et al., 2010). However, copper ions are naturally occurred in water in the water delivery system because of corrosion of the copper pipes and fitting. Therefore, copper ion indirectly will effect human health and cause disease such as thalassemia, yellow atrophy of liver, tuberculosis and carcinomas (Farhan and Khadom, 2015).

The present study was carried out to investigate the adsorption breakthrough curve of copper ion on immobilized *S.cerevisiae* in a packed-bed column

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under the effect of flow rate, bed height and initial concentration.

2. Material and methods

2.1. Preparation of Biosorbent

Mauri-Pan instant baker's yeast was used as a biosorbent. 2% (w/v) sodium alginate was dissolved in distilled water and mixed with 12% (w/v) Mauri-Pan instant baker's yeast. The mixture was stirred on magnetic stirred until get uniform slurry. Then, the slurry was dripped into 1.5% (w/v) calcium chloride solutions using a syringe and beads were immediately formed in the solution as shown in Fig. 1.

2.2. Preparation of stock solution

1000 ppm of copper (II) solution was prepared as an aqueous stock solution using copper (II) sulfate by dissolving into 1000mL distilled water. The solution was diluted prepare the working solutions and pH of influent solution was adjusted to 5.5 with 1.0 M hydrochloric acid and 1.0 M sodium hydroxide.

Fig. 1: Immobilized *S.cerevisiae* in calcium chloride solution

2.3. SEM-EDX characterization

Scanning electron microscopy with energy dispersive X-ray spectroscopy (SEM/EDX) was used in order to analyze surface characteristics and elemental information of immobilized *S.cerevisiae* before and after the process of biosorption. An image of immobilized *S.cerevisiae*surface can be seen from SEM analysis and EDX analysis will give elemental and composition information (Thippeswamy et al., 2014).

2.4. Column experiments

Continuous studies were conducted in a glass column having inner diameter 1.2cm and height 20cm. The column was filled with glass beads at the bottom and top of the column in order to provide a uniformed flow of the solution through the column. Schematic diagram of biosorption of copper (II) in packed-bed column is shown as Fig. 2.

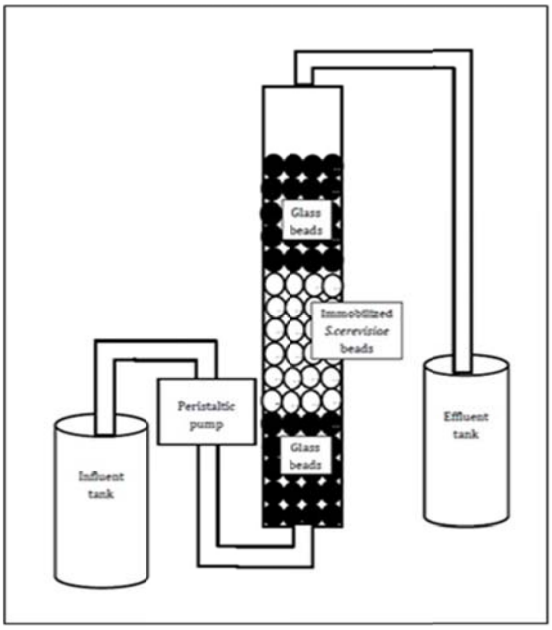


Fig. 2: Schematic diagram of biosorption of copper (II) in packed-bed column

An initial concentration of 300 mg/L copper (II) was pumped upward through the column at a desired flow rate with a peristaltic pump (Masterflex, Cole-Parmer Instrument Co., US) and pH maintained at 5.5. The effluent samples were collected at regular time intervals and analyzed for copper (II) concentration using inductively coupled plasma (Perkin-Elmer Optima 7000DV).

2.5. Analysis of column data

To analyzed the dynamic removal of copper (II) in column mode, breakthrough curves show the loading behavior of copper (II) to be removed from the solution in a packed-bed column and is usually expressed in terms of adsorbed concentration (*Cad*), inlet concentration (*Co*), outlet concentration (*Ct*) or normalized concentration defined as the ratio of outlet concentration of inlet concentration (*Ct/Co*) as a function of time.

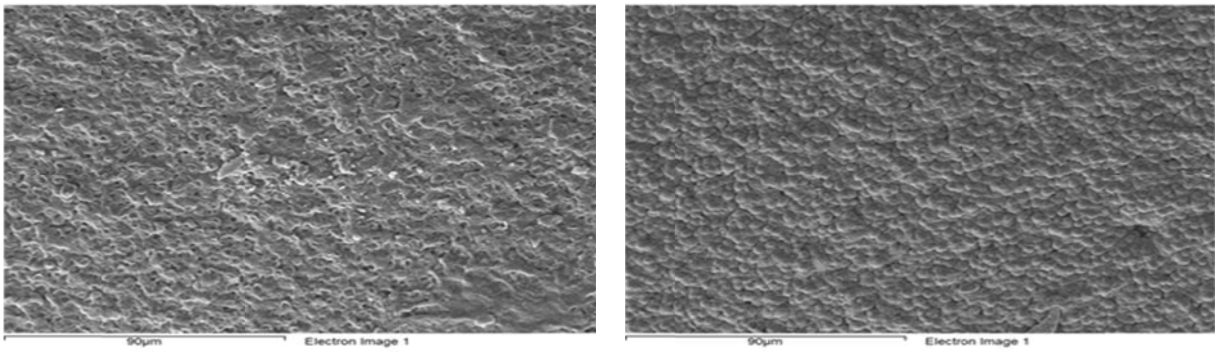
3. Results and discussion

3.1. SEM analysis

SEM images of immobilized *S.cerevisiae* before and after biosorption were shown in Fig. 3. The image from Fig. 3(a) and 3(b) reveals the structures of immobilized *S.cerevisiae* slightly change before and after biosorption process. It clearly showed in Fig. 3(a), a lots of tiny interspace structure distributing on the surface of the immobilized *S.cereviaie* before biosorption process which contributed to more surface area of copper (II) to adhere on surface of immobilized *S.cereviaie* (Liu et al.,2013). Fig. 3(b) showed the surface of immobilized *S.cerevisiae* after biosoprtion of copper (II) become smoother and protrusions compared before bosorption. The surface structure change showed that biosorption of copper (II) occur on immobilized *S.cerevisia* (Nguyen et al., 2015).

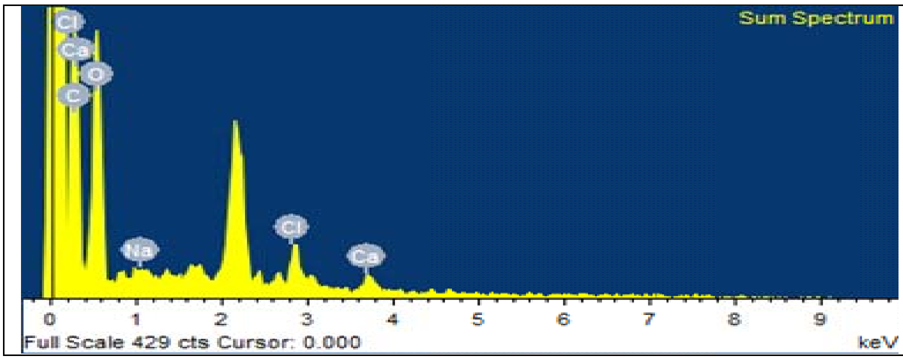
3.2. EDX analysis

EDX analysis was used to reveal that copper (II) has bind on the surface of immobilized *S.cerevisiae*. Fig. 4(a) and 4(b) showed the element composition have been detected before and after biosorption of copper on immobilized *S.cerevisiae*. The elements showed in Fig. 4(a) were used as control elements to compare with elements reveals after biosorption. The results clearly reveals in Fig. 4(b) that copper (II) was adsorb by immobilized S.cerevisiae (Nguyen et al, 2015).



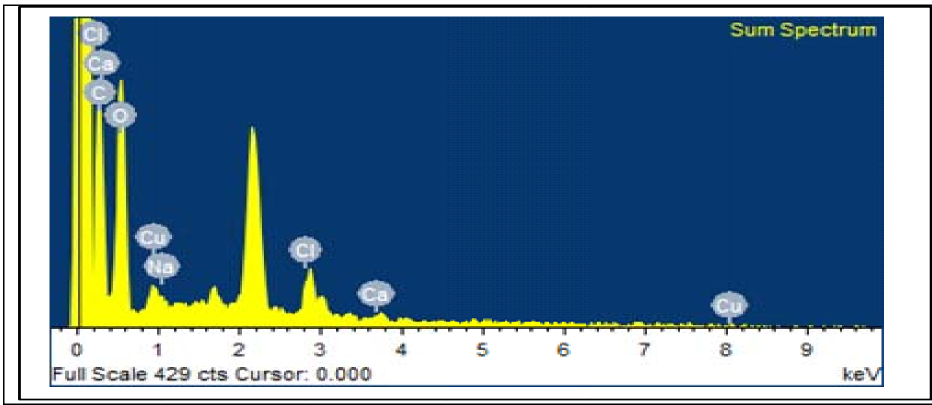
a b

Fig. 3: SEM images of immobilized *S.cerevisiae* before and after biosorption



Element	Weight%	Atomic%
C	32.16	40.56
O	59.00	55.86
Na	0.28	0.19
Cl	3.18	1.36
Ca	5.37	2.03
Totals	100.00	

Fig. 4 (a): EDX images of immobilized *S.cerevisiae* before biosorption



Element	Weight%	Atomic%
C	20.10	27.25
O	66.75	67.95
Na	1.53	1.09
Cl	0.36	0.17
Ca	4.40	1.79
Cu	6.85	1.76
Totals	100.00	

Fig. 4(b): EDX images of immobilized *S.cerevisiae* after biosorption

3.3. Effect of inlet concentration

The sorption breakthrough curves obtained by changing inlet copper (II) concentration from 100 to 300 mg/L with constant flow rate (12mL/min) and bed height (12cm). Fig. 5 showed a lowest inlet concentration gave a later breakthrough curve and the treated volume was the greatest at the lowest inlet concentration since the lower concentration gradient caused a slower transport due to a decreased diffusion coefficient or decreased the mass transfer coefficient. It was observed that the adsorbent quickly saturated at a highest inlet concentration since the equilibrium uptake of copper was high at highest inlet concentration (Nasuha et al., 2014). The breakthrough time was found to decrease with increasing inlet concentration as the binding sites became quickly saturated in the column (Chowdhury et al., 2012).

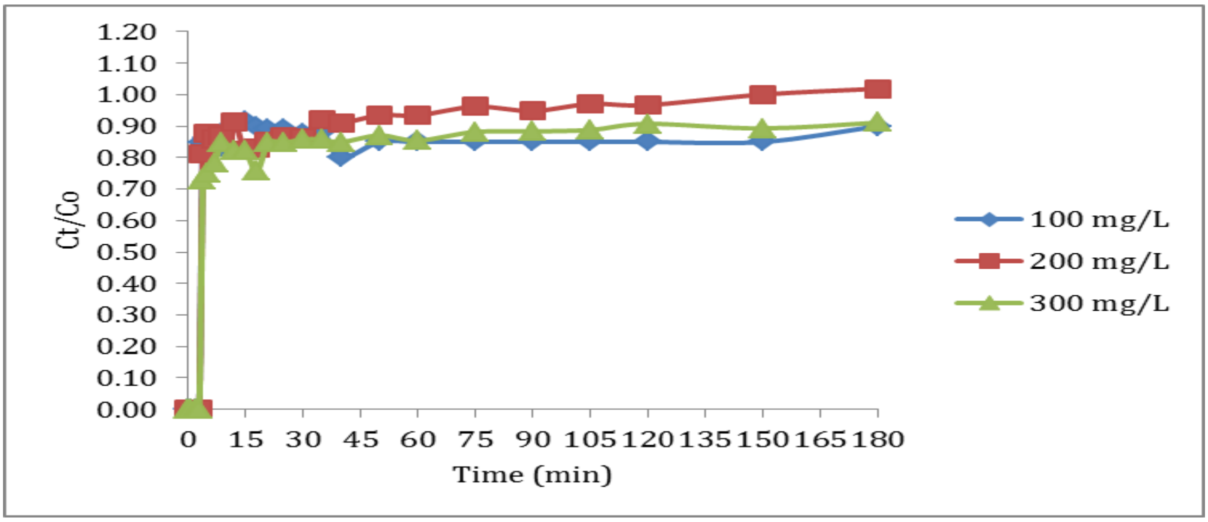


Fig. 5: Breakthrough curves for copper (II) biosorption onto *S.cerevisiae* at different initial concentration (Bed height: 12cm; flow rate: 12mL/min)

3.4. Effect of bed height

The effect of bed height on copper (II) absorption by immobilized *S.cerevisiae* was studied by varying the bed height from 4 to 12 cm maintaining the flow rate and inlet copper (II) concentration at 12 mL/min and 100mg/L respectively. It can be observed from Fig. 6 that high volume of copper (II) treated was at the high bed height. This was due to a relatively small amount of adsorbent in a shorter bed, an increase in the bed height resulted in an increase of equilibrium time of biosorption. The increase in biosorption amount with the amount adsorbent could be due to an increase in the surface area and the availability of the more binding sites on the adsorbent (Chhikara et al., 2010). As expected, breakthrough time increased with increasing bed height. Kumar et al. (2011) claimed that amount of heavy metal treated depend on amount of the adsorbent used during the adsorption process.

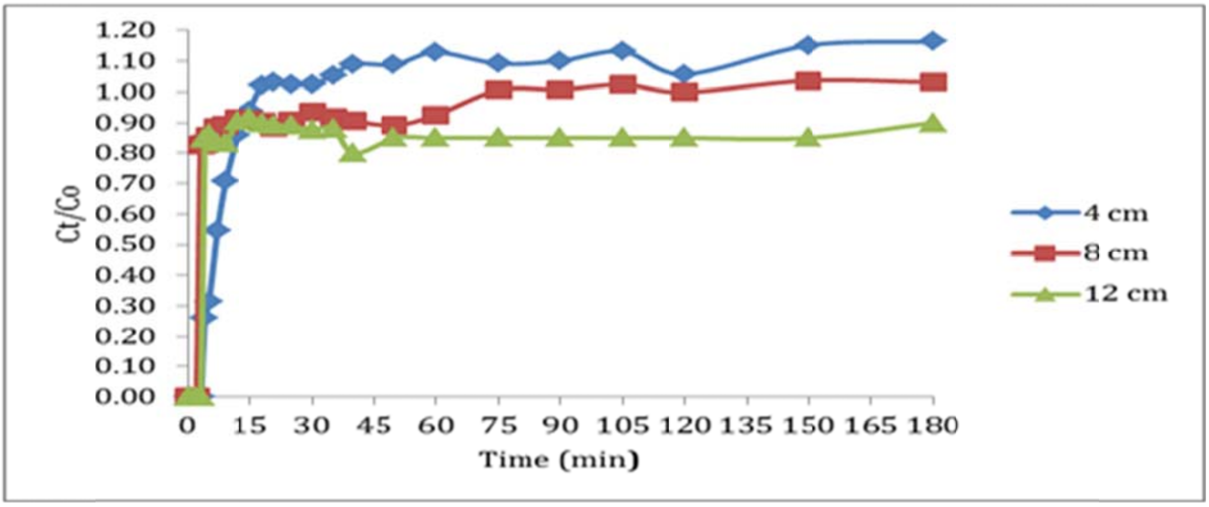


Fig. 6: Breakthrough curves for copper (II) biosorption onto *S.cerevisiae* at different bed height (Initial copper (II) concentration: 100mg/L; flow rate: 12mL/min)

3.5. Effect of flow rate

The effect of flow rates on copper (II) biosorption in packed bed column was investigated by varying the flow rate from 12 to 36 mL/min while bed height and initial concentration constant at 12cm and 100mg/L. Fig. 7 showed that the breakthrough time decreased with increasing in flow rate. This is due to insufficient time for solute inside the column and diffusion limitations of solute into pores of sorbent at high flow rate (Kumar et al., 2011). The higher removal of copper (II) biosorption was indicated at lowest flow rate which copper (II) had more time to be contact with adsorbent.

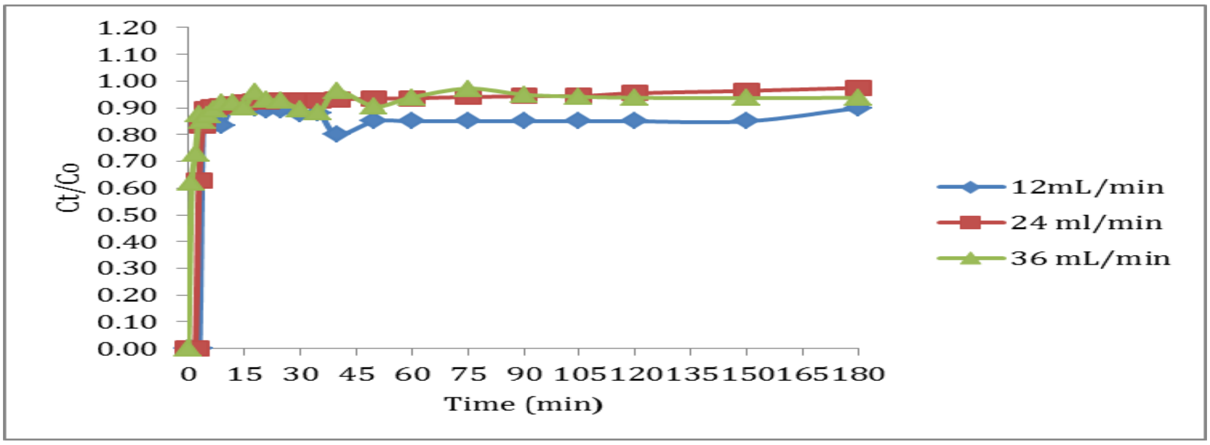


Fig. 7: Breakthrough curves for copper (II) biosorption onto *S.cerevisiae* at different flow rate (Initial copper (II) concentration: 100 mg/L; bed height: 12cm)

3.6. Regeneration studies

The regeneration of copper (II) were repeated three times using 1.0 mol/L HCL solution and the result was shown as Fig. 8. The results showed regeneration efficiency was decreased from first cycle until 3rd cycle. Moreover, it was observed that the breakthrough time and performance of biosorption decreased with each regeneration. This is due to gradual deterioration of adsorbent and adverse effect of eluting agent on sorption sites (Kumar et al., 2011).

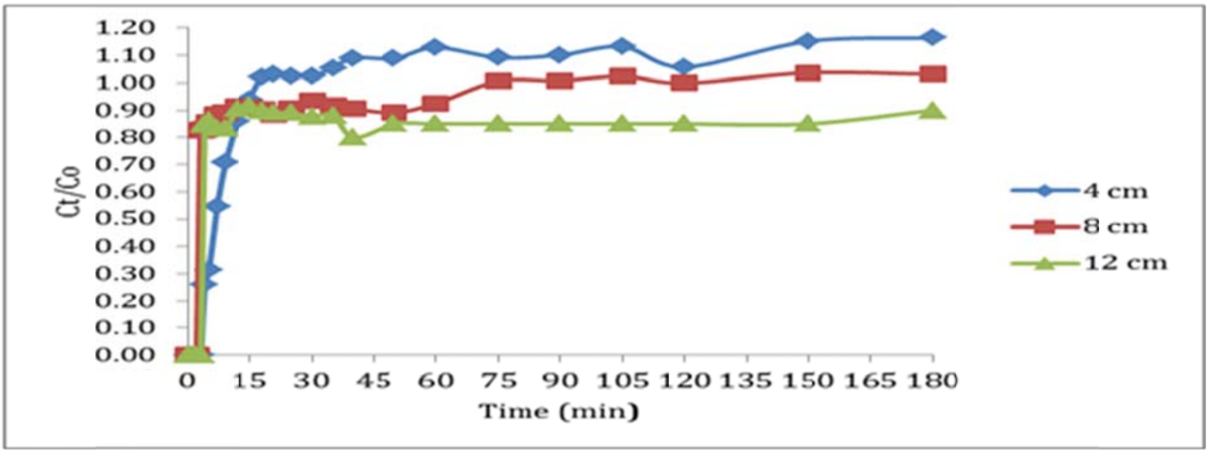


Fig. 8: Regeneration immobilized *S.cerevisiae* for copper (II) biosorption

4. Conclusions

The study showed that immobilized *S.cerevisiae* as an effective and potential biosorbent for the removal of copper (II) from aqueous solution in a continuous packed-bed column. The study revealed the importance of initial concentration, bed height and flow rate on copper (II) biosorption during continuous process. It was found that the adsorbent faster saturated at the high inlet concentration and the breakthrough time decreased with increasing initial concentration of copper (II) biosorption onto immobilized *S.cerevisiae.* The result indicated the high copper removal at the maximum bed height and the lowest flow rate which at low flow rate copper (II) had more time to be contact with adsorbent. The regeneration efficiency of immobilized *S.cerevisiae* was observed up to 3 cycles. These observations indicated that regeneration of adsorbent in biosorption process makes the process more economical.

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