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Biosorption of Ternary Cadmium, Nickel and Cobalt Ions from Aqueous Solution onto *Saccharomyces cerevisiae* Cells: Batch and Column Studies

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ABSTRACT

The aim of this study was to remove cadmium, nickel and cobalt ions from aqueous solutions using yeast, *Saccharomyces cerevisiae*, pre-treated with ethanol. The cells are immobilized using polysulfone in both batch and continuous systems. To optimize the adsorption capacity of the yeast biomass, a batch system was used to investigate the effects of initial pH, initial biomass dose and initial metal ion concentration. Optimum conditions were obtained at pH 8 with a biomass of and ion concentrations of 8 and 100 mg L^{-1}, respectively. Under these conditions, the maximum uptake capacity was 3.1 mg g^{-1} for cadmium, 1.2 mg g^{-1} for nickel and 0.68 mg g^{-1} for cobalt. To estimate the biomass uptake capacity of the ethanol pretreated *S. cerevisiae*, the isothermal Langmuir model was found to be a better fit with a correlation coefficient of $R^2 <0.964$. Factors observed affecting biomass ion adsorption in the continuous system are initial concentration of single or multi-metallic ions, the column height of bead system and the input flow rate. The optimum conditions for the continuous system were determined at pH 8, initial metal ion concentration of 100 mg L^{-1}, bead column height of 25 cm and the input flow rate of 1 mL min^{-1}. The calculated uptake capacity of the metal ions was 3.74 mg g^{-1} for cadmium, 1.57 mg g^{-1} for nickel and 1.56 mg g^{-1} for cobalt. The Thomas model ($0.9524<R^2<0.9957$) was shown to be more consistent compared with the test results. Polysulfone immobilization of yeast was found to increase removal by 48% compared to non-immobilized ethanol pr-treated yeast.

Keywords: Biosorption, *Saccharomyces Cerevisiae*, Ethanol, Polysulfone, Cadmium, Nickel, Cobalt

1. INTRODUCTION

Heavy metals are very important contaminants of surface and underground water sources and are at least 5 times heavier than water. They are often effluents from various industries such as metal treatment, mining or pulp production and enter water systems via untreated waste (Zvinowanda *et al*., 2010). They are elements with high stability and the ability to bioaccumulate in the food chain. These metals are extremely toxic and harmful even at low concentrations. Thus heavy metal treatment of waste is important in preventing these elements entering our food chains (Hussain *et al*., 2009). There are several methods for removing heavy metals from wastewater, such as using inverse-phosphor, electrochemical, ion balance, chemical deposition. Each of these methods demonstrates high purification capability, especially for metal concentrations between 0.01-0.1 g L^{-1}. However, these methods are expensive and may produce harmful by-products (Zvinowanda *et al*., 2010). Thus, further research for cheaper removal methods using materials such as coal, fly ash, agricultural waste materials and biosorbents are in demand.

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By using heavy metal biosorption methods, old chemical and physical methods used in wastewater purification can be improved (Wang and Chen, 2006). Biosorption technology is applied using cheap organic materials to remove heavy metals from aqueous environments (Vijayaraghavan *et al*., 2009). The living or non-living biomass of various materials such as bacteria, fungi, algae and yeasts are commonly used (Pokhrel and Viraraghavan, 2008). Non-living biomass has shown special advantages in comparison with living microorganisms and can be stored for long periods (Vijayaraghavan *et al*., 2005; Amini *et al*., 2013). They neither respond to the toxicity of heavy metals nor need nutrients. In addition, physical and chemical methods to pre-treated and preserve the biomass will improve its adsorption qualities. Due to their protozoan nature and high growth rates, yeast cells are ideal adsorbents for heavy metals adsorption. Yeast cells are easy to grow cheaply thus an accessible source of biomass with high potential to adsorb residue at low pH (Wang and Chen, 2006; Vijayaraghavan *et al*., 2009; Park *et al*., 2003; Vasudevan *et al*., 2003; Vimala *et al*., 2011).

This study aims to remove the heavy metals of cadmium, nickel and cobalt using *S. cerevisiae*, pre-treated by 70% ethanol and immobilized with polysulfone polymer. Various variables affecting adsorption were investigated in both continuous and batch systems.

2. MATERIALS AND METHODS

2.1. Biomass Preparation and Pre-Treatment

S. cerevisiae PTCC 5010 was prepared and cultured in a sterile medium from Researched Technology Department of the Ministry of Sciences from the Persian Type Culture collection in form of freez dry. The growth medium consisted of 7% (w/v) glucose, 0.1% (w/v) yeast extract, 0.9% (w/v) $(NH_4)_2SO_4$, 0.25% (w/v) $MgSO_4$, 0.1% (w/v) KH_2PO_4 and 0.02% (w/v) K_2HPO_4. Sterilisation was performed for 20 min at 1.5 atm and 121°C in an autoclave. The medium temperature was adjusted to ambient temperature (25°C) for inoculation. *S. cerevisiae* cells were grown in an orbital shaker at 200 rpm for 3 days after which the cells were centrifuged and filtered with a 0.45 mm filter (Park *et al*., 2003; Ghorbani *et al*., 2008). Yeast biomass was dried in an oven at 100°C for 24 h (Ozer and Ozer, 2003) and screened by a 100 mm mesh sieve accordingly (Ghorbani *et al*., 2008). The cells are treated by ethanol by soaking dried cells in 100 mL ethanol solution of 700 g L^{-1} for 20 min at room temperature (25°C). After the treatment, yeast cells are centrifuged at 3600 rpm for 10 min and the ethanol solution removed. The separated biomass was washed several times with deionised water to remove the remaining ethanol and any adsorbed nutritive ions. Centrifuging the washed yeast the separated biomass was heated at 100°C for 16 h, crushed then screened by a 100 mesh sieve (Goksungur *et al*., 2005). Crushing is vital to homogenise yeast biomass to prevent biomass from accumulation and thus increase the adsorption capacity. The crushed biomass was chilled for subsequent use for adsorption studies (Ghorbani *et al*., 2008).

2.2. Immobilization of Biomass

For immobilization studies, a 100 mL of N, N-Dimethylformamide (DMF) solution from pre-treated biomass was combined with 7 g polysulfone (Hasan and Srivastava, 2009). The beaker was immediately covered to prevent evaporation of DMF and put on a magnetic stirrer for 16 hrs to completely dissolve the polysulfone in DMF and achieve a stable slurry (Munagapati *et al*., 2010). The slurry was dripped carefully into a glass jar containing deionized water using a syringe (10 mL). The obtained beads were dispersed by mixer for 8 hrs. After which the beads were dried out at room conditions for 2-3 days and isolated manually to be stored at 4°C (Hasan and Srivastava, 2009).

2.3. Metals Solution and Analysis

Stock solution was prepared by diluting 1000 mg L^{-1} of solution of chloride salts (1.631 g of $CdCl_2$), (4.05 g of $NiCl_2 \cdot 6\,H_2O$) and (4.037 g of $CoCl_2 \cdot 6\,H_2O$) (Merck) in deionised water. The obtained solution was used to prepare different concentrations and standards needed for the tests. The samples of different concentrations of metal solutions were examined using atomic absorption spectrometry (AAS, Philips, PU9400, UK) (Bhatnagar *et al*., 2010). The initial pH was adjusted by addition of 2N HCl and 2N NaOH solutions.

2.4. Batch Adsorption Studies

In batch adsorption tests, the effects of pH, the initial metal ion concentration and the volume of pre-treated biomass were studied. All the tests were performed in 250 mL Erlenmeyer flask containing 100 ml of metal solution shaking at a speed of 200 rpm. Sampling was performed at the intervals of 0, 5, 10, 20, 40 and 80 min until equilibrium time was reached. Each sample solution is filtered via a 0.45μm syringe filter and analyzed to determine the concentration of metal ions.

All the tests were performed at room temperature (25°C). Uptake capacity of the biomass was calculated using Equation (1):

$$q_e = \frac{V}{M}(C_0 - C_e) \tag{1}$$

where, q_e is the amount of adsorbed metal ions of the adsorbent (mg/L), V is the volume of metal solution in contact with the adsorbents (ml); C_o and C_e are the initial concentration and equilibrium concentration of metals in the solution (mg/L) respectively and finally M is the amount of the dry adsorbent (g) (Wang and Chen, 2006). Also, the removal percentage (R) is calculated according to Equation (2) in which percentage of metal can be sorbed by biomass in solution:

$$R = \frac{C_0 - C_e}{C_0} \times 100 \tag{2}$$

2.5. Isotherm Modeling

It is important to analyze the equilibrium data to build an equation that can be used to compare the biosorbents under different conditions. The isotherm models use a wide range of parameters to examine the relationship between the biosorption capacity and adsorbent concentration at equilibrium. Langmuir and Freundlich are two different adsorption isotherm models widely used for data estimation.

2.6. Langmuir Isotherm

Langmuir adsorption isotherm was successfully used for the biosorption process of heavy metal ions. The primary hypothesis of Langmuir theory is that at particular homogeneous sites, the biosorption process occurs in the adsorbent. This model can be written in a nonlinear form as show in Equation 3 (Munagapati *et al*., 2010):

$$q_e = \frac{q_m k_L C_e}{1 + k_L C_e} \tag{3}$$

where, q_e is the metal ion concentration in the adsorbent (mg/g), C_e is the concentration of the equilibrium metal ions in the solution (mg/L), q_m is the monolayer adsorption capacity of adsorbent (mg/g) and k_L is the ratio of Langmuir adsorption constant (L/mg) to the free energy of uptake. Langmuir constant k_L can be measured using Hall isolation factor (R_L) to determine the affinity of the adsorbent to the adsorbate (Akar *et al*., 2009). The value of R_L can be calculated using the following Equation (4):

$$R_L = \frac{1}{1 + k_L C_o} \tag{4}$$

where, C_o is the highest initial concentration of the adsorbate (mg/L).

2.7. Freundlich Model

The hypothesis of Freundlich models is heterogeneous adsorption. This model is in accordant with the following Equation (5):

$$q_e = K_F C_e^{1/n} \tag{5}$$

where, K_F is a constant between biosorption capacities and n is an experimental parameter between intensity of biosorption (Wang and Chen, 2006).

2.8. Column Adsorption Study

The pre-treated immobilized biomass was packed within a glass column with an internal diameter of 2 cm and height of 40 cm. The bed dimensions are 126/6 mL. Glass wool (1cm thick) was put at the top and the bottom of the column to allow the liquid to enter the column, spread evenly and prevent the beads from floating. According to the tests, the beads are packed in columns of different heights. The inlet tube was placed at the bottom of the column and the metal solution was pumped into the column using a peristaltic pump (Heidolph 5101, Germany).

All the continuous system tests were performed at pH 8, which was determined based on the batch system studies. All tests were conducted at room temperature (25°C). The system variables, including the concentration of the stock solution were 100, 50, 20 and 10 mg L^{-1} for ternary metal ion solutions and 50 mg L^{-1} for a single metal ion. The bead column height for this variable was 25 cm and the flow rate was 1 mL min^{-1}.

The second variable was the height of the bead in the column which was studied at the heights of 20, 25 and 30 (9 g) cm in which the flow rate and the metal solution concentration were 1 mL min^{-1} and 10 mg L^{-1}, respectively. The last variable was the flow rate of the input liquid to the column which was selected as 0.5, 1, 2 and 4 mL min^{-1}. In this case, the bead column height and metal ion concentration were 25 cm and 100 mg L^{-1}, respectively. Sampling was continued in short intervals until exhaustion. The subject of this study was to examine the breakthrough analysis of the biosorption of Cd (II), Ni (II) and Co (II) by biomass beads of immobilized *S. cerevisiae* in the column. In addition, Thomas and Yan (1988) models were used to describe the kinetic energy in the fixed bed column.

2.9. Evaluation of Break through Curves and Mass Transfer in Packed Column

Breakthrough graphs in the test are required to examine the numerical values. MATLAB (version 7) software was used for breakthrough and the maximum uptake capacity measurements.

The breakthrough and the maximum uptake capacity were calculated by measuring the area under the breakthrough curve at the breakthrough point $C_e = 0.05C_o$ and the full exhaustion point (Naddafi *et al*., 2007) as shown in Equation (6):

$$q = \frac{m_{inf} - m_{eff} - m_{pore}}{m_{biomass}} = \frac{C_0 V_c - Q\int_0^t C_{eff}(t)\,dt - C_0 V_v}{m_{biomass}} \qquad (6)$$

where, q is the mass of the metal ions adsorbed onto the biomass (mg/mg), m_{inf} is the total mass of the metal sent into the column (mg), m_{eff} is the total mass of the metal emitted from the column (mg), m_{pore} is the total mass of the metal remaining in the biomass pores (adsorbent) which has not been adsorbed (mg), $m_{biomass}$ is the mass of biomass (adsorbent) (g), C_0 is the initial metal concentration (mg/L) and V_c is the total volume of the ions sent into the column (L), V_v is the throughout volume of metal ions (L), Q is the input flow rate (ml/min), t is the total working time of the column (min) and $C_{eff}(t)$ is the output concentration as a function of time (mg/L.min).

The Mass Transfer Zone (MTZ) is one of the widely used parameters to examine the effects of the column adsorption height. To determine the length of the adsorbent zone in the column, MTZ can be calculated from the following formula Equation (7):

$$MTZ(cm) = L\frac{t_e - t_b}{t_e} \qquad (7)$$

where, L presents the closed height (cm), t_b is the time (min) required to reach the breakthrough point or $C_{eff}/C_o = 0.05$ and t_e is the time (min) required to reach the exhaustion point or $C_{eff}/C_o = 0.95$ (Apiratikul and Pavasant, 2008).

Several mathematical models have been proposed, with the Thomas model and Yan model selected to describe the biosorption of a fixed-bed column. Thomas model used is expressed as follows:

$$\frac{C_{eff}}{C_0} = \frac{1}{1 + \exp\left(\frac{k_{th}}{Q}\left(mq_{th} - C_0 V\right)\right)} \qquad (8)$$

where, C_{eff} is the heavy metal ion concentration (mg/L), C_o is the initial concentration of heavy metal ions (mg/L), k_{th} is Thomas constant rate ml/min, q_{th} is the maximum metal adsorbed by biomass in mg/g, m is the mass of adsorbent (g), V is the throughput volume (L) and Q is the flow rate ml/min (Yan and Viraraghavan, 2001). By setting $a = 1000mq_{th}/QC_o$ and $a = 1/k_{th}C_0$, Equation (8) can be written as:

$$\frac{C_{eff}}{C_0} = \frac{1}{1 + \exp\left(-(t-a)/b\right)} \qquad (9)$$

Yan is an empirical model that can overcome the Thomas model weakness in the prediction of the throughput concentration at time zero. The Yan model expressed in Equation (10) describes the area under the breakthrough curves in a fixed bed column (Yan and Viraraghavan, 2001):

$$\frac{C_{eff}}{C_0} = 1 - \frac{1}{1 + \left(\frac{t}{b}\right)^a} \qquad (10)$$

where, $b = q_y mQ/1000C_o$ are the constant rates of kinetic energy flow rate (l/h/µg), q_y is the maximum adsorption by the adsorbent (mg/g) estimated using the model (Pokhrel and Viraraghavan, 2008). Equation (10) expresses the expansion of Equation 9 and t conditions Equation 11:

$$\frac{C_{eff}}{C_0} = 1 - \frac{1}{1 + \left(\frac{QC_0 t}{q_y m}\right)^a} \qquad (11)$$

The column flow data were evaluated using Equation (9 and 10) and nonlinear correlation analysis performed via Sigma Plot 12.0 software.

3. RESULTS AND DISCUSSION

3.1. Batch Biosorption

3.1.1. Effect of pH

Many studies have shown that the most important parameter in the removal of heavy metals by adsorbent is the initial pH of the solution.

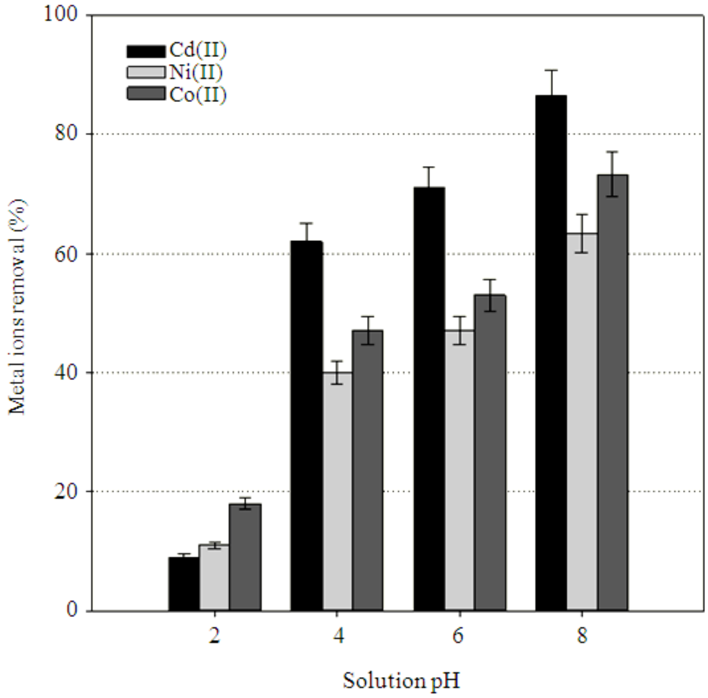

Fig. 1. Uptake capacity metal ions at verify pH with biomass doses 8 g L^{-1} and co-ions metal concentration 10 mg L^{-1}

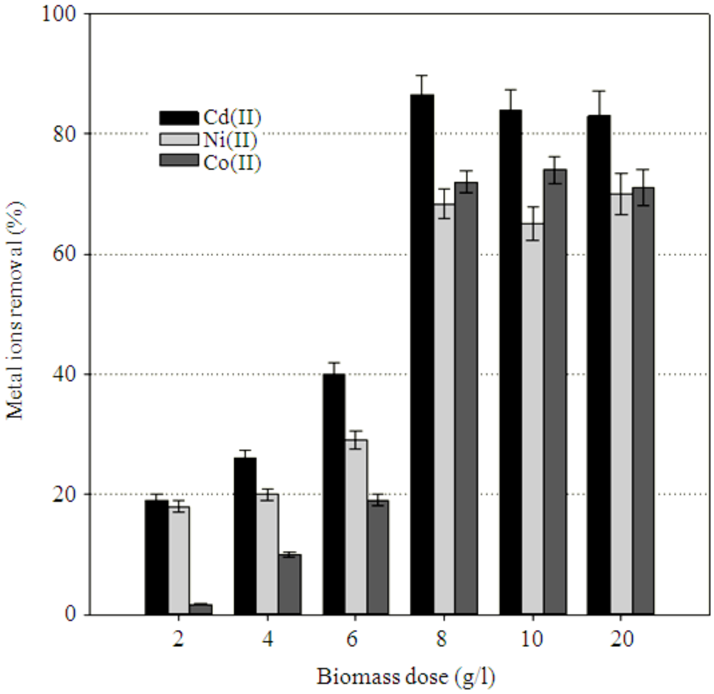

Fig. 2. Yeast uptake capacity at different biomass doses by pH 8 and co-ions metal concentration 10 mg L^{-1}

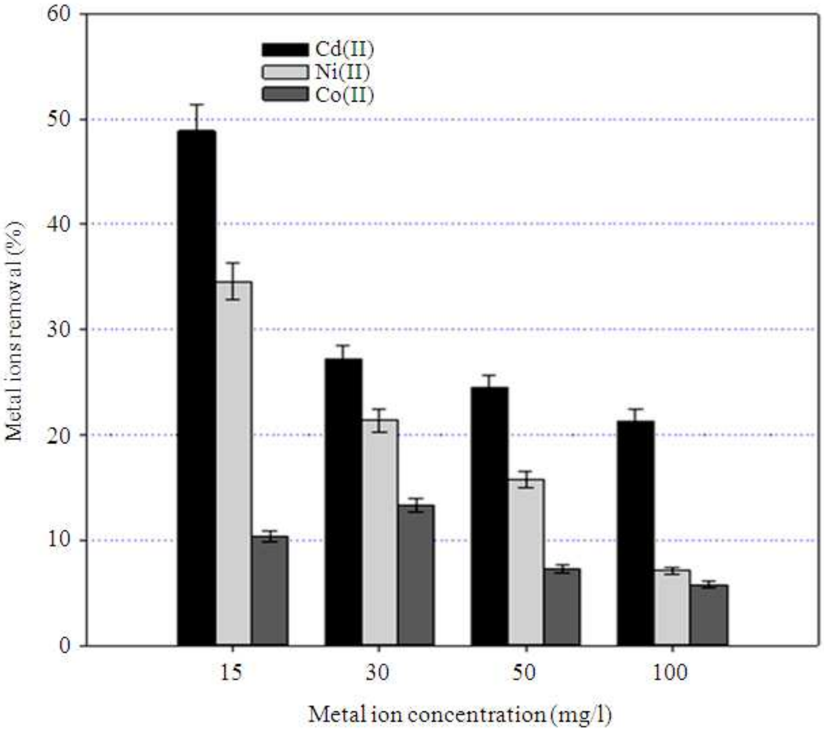

Fig. 3. Removal percentage of different concentrations of co-ions metal at biomass dose 8 g L^{-1} and pH 8

The results similarly reflect that the pH of the solution can significantly affect the biosorption (Liu *et al*., 2009). With the constant 8 g L^{-1} of the biomass and 10 mg L^{-1} of the metallic ions, effect of varying initial pH on the adsorption of metal ions at pH 2, 4, 6 and 8 on the adsorption of cadmium, nickel and cobalt was recorded.

The adsorption of metals is correlated to functional groups adsorption of protein (Park *et al*., 2003; Baysal *et al*., 2009). At very low pH the total surface charge of the cells becomes positive, the competing metal cations and protons for binding sites on the cell wall consequently reduces metal adsorption (Ghorbani *et al*., 2008; Amini *et al*., 2008). It is believed that the existing groups in the cell wall have a high correlation with H_3O^+ which limits the adsorption of metal ions as a result of repulsive forces (Wang and Chen, 2006; Goksungur *et al*., 2005). The results of 11.4% for cadmium, 14.1% for nickel and 21% for cobalt showed that at pH 2 very little removal was achieved. By increasing the pH from 2 to 8, the removal percentage was increased and reached 86.5%, 63.3% and 73.4 for cadmium, nickel and cobalt, respectively. The uptake capacity also increases according to the values presented in **Fig. 1**. At high pH, the decrease in H^+ ions causes the surface of the cell walls to be covered with negative charges. This then generates conductive conditions for carboxylic, phosphate, hydroxyl and amino acid groups. All of which easily react with metal ions therefore, resulting in the increased adsorption of metal ions (Liu *et al*., 2009; Amini *et al*., 2008). However at pH higher than 8, adsorption is inhibited as metal ions are deposited on the bead surface due to the high concentration of OH^- ions (Dursun *et al*., 2006). The test results were consistent with that of other studies (Park *et al*., 2003; Vasudevan *et al*., 2003; Ghorbani *et al*., 2008; Ozer and Ozer, 2003; Goksungur *et al*., 2005).

3.2. The Effect of Biosorbent Dose

Six varying doses of biomass were selected and added to samples of 10 mg L^{-1} ternary metal ions solution at pH 8. By increasing the biomass dose from 2 to 20 g L^{-1}, the removal percentage for all three metals rose. The lowest removal percentage was achieved at biomass concentration of 2 g L^{-1} and the highest removal percentage obtained at 20 g L^{-1} biomass which was 83% for cadmium, 70% for nickel and 61% for cobalt. These results indicate that by increasing biomass dosage, the availability of active adsorption sites grew and thereby, removal increased (Vijayaraghavan *et al*., 2009; Baysal *et al*., 2009). On the other hand, the uptake capacity will decrease with increasing biomass dose. The optimum uptake capacity of the biomass was calculated at the equilibrium concentration. Its maximum value in the biomass dose of 8 g L^{-1} was 1.8 mg g^{-1} for Cadmium, 1.8 mg g^{-1} for nickel and 1.05 mg g^{-1} for cobalt. **Figure 2** shows the uptake capacity of the three metals with different amounts of biomass dose. Reduction of the uptake capacity for a high dose of biomass is related to the unsaturation of the active sites during the adsorption process. Hence due to the increased biomass, the amount of active sites unsaturated was increased (Munagapati *et al*., 2010; Baysal *et al*., 2009; Han *et al*., 2006). Similar results were reported in other independent studies (Vijayaraghavan *et al*., 2005; Munagapati *et al*., 2010; Apiratikul and Pavasant, 2008; Baysal *et al*., 2009; Han *et al*., 2006).

3.3. Effect of Initial Metal Ion Concentration

The effect of initial concentration on the removal of the three metals was studied in medium at room temperature (25°C), pH 8 and fixed biomass concentration of 8 g L^{-1} (**Fig. 3**). The initial metal ion concentration was investigated in the concentration range of 10-100 mg L^{-1}. The results showed that the removal percentage decreases with increasing metal concentration. Lowest removal value was observed at the concentration of 100 mg L^{-1} and its highest value was observed at 15 mg L^{-1}. This data is in disagreement with the results obtained in the uptake capacity calculations. By increasing the initial metal ion concentration, adsorption will also increase. The initial metal ions concentration enhances the driving forces and the uptake capacity. At low concentrations, all metal ions react with the active sites in the adsorbent with each active site surrounded by more metal ions. Therefore, uptake capacity will increase rapidly initially. At high concentrations, saturated adsorption sites result in approximately constant uptake capacity (Akar *et al*., 2009; Baysal *et al*., 2009; Han *et al*., 2006). The highest uptake capacity was found to be 3.19 mg mg^{-1} for cadmium, 1.28 mg mg^{-1} for nickel and 0.61 mg mg^{-1} for cobalt.

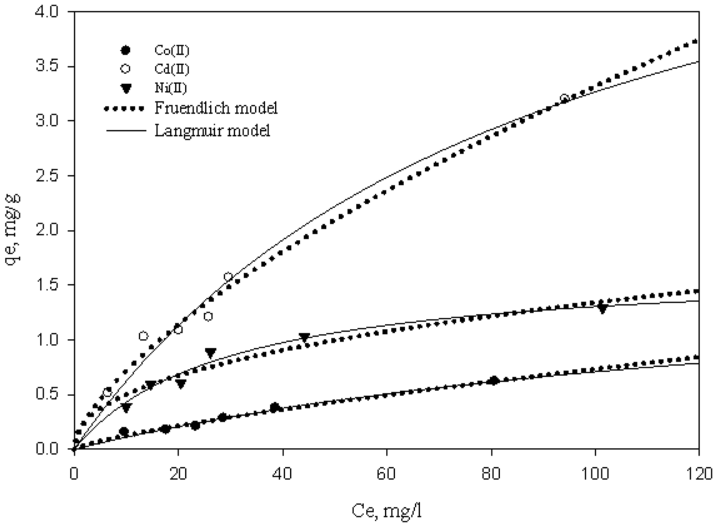

Fig. 4. Isotherms Langmuir and Freundlich models at initial pH 8 and biomass dose 8 g L^{-1}

Table 1. Parameters isotherm langmuir and freundlich models for ions metal

	Langmuirr isotherm				Freundlich isotherm		
Metals	q_m, mg/g	b, l/mg	R^2	R_L^*	K_f	n	R^2
Ni(II)	1.683	0.034	0.964	0.225	0.186	2.334	0.914
Co(II)	1.768	0.0067	0.975	0.598	0.021	1.302	0. 979
Cd(II)	1.179	0.011	0.978	0.471	0.155	1.506	0.987

*R_L calculated for initial concentration 100 mg L^{-1} (C_o)

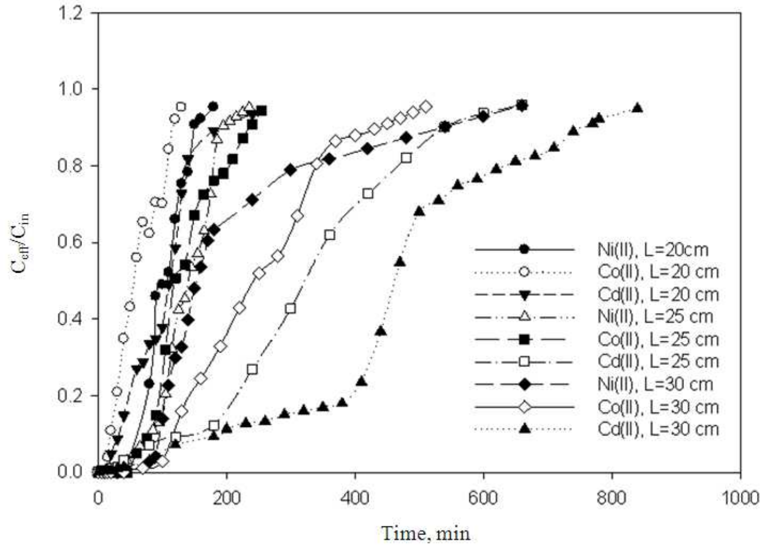

Fig. 5. Breakthrough curve at the different heights of the bead column and constant flow rate 1 ml/min, ion concentration 100 mg L^{-1} and pH 8

3.4. Isotherm Modeling

Adsorption isotherms are important in describing the mutual relationship between adsorbate and adsorbent and identifying critical conditions to be optimized. The adsorption isotherm index is described by constant values that represent the properties and the adsorbent surface affinity. To determine the behavior of the adsorption system, the uptake capacity and the conformity of the adsorption process with isotherm models, the changes of the value q_e was studied in terms of equilibrium concentration of metal ions. For this purpose, six solutions with different amounts of ternary metal ions of all three metals (i.e. cadmium, nickel and cobalt) were prepared at pH 8 with the biomass concentration of 8 g L^{-1}. In the applied isotherm models, Freundlich and Langmuir, which respectively indicate single-layer adsorption and heterogeneity was used. Both Freundlich and Langmuir models were in good accordance with the test data. **Figure 4** reflects the test data consistency with isotherm models for all three metals. **Table 1** shows the fixed values in the Freundlich and Langmuir isotherm models. Referring to the R^2 values, the Langmuir model was comparatively more consistent with the adsorption process than Freundlich (Goksungur *et al*., 2005; Akar *et al*., 2009). The maximum value of metal adsorption (q_m) for all three metals was found in the order: cobalt > nickel > cadmium. At the maximum initial metal ions concentration (100 mg L^{-1}), the value of R_L was measured between 0-1 in Langmuir model, which represents the optimal adsorption by the adsorbent and was measured for each metal in these tests. The results showed that nickel is more capable of forming complexes with the yeast biomass than the other two metals. Freundlich constants n and K_F indicate uptake capacity and adsorption density, respectively and their high value represents the easy adsorption of metal ions from the aqueous solution and favorable adsorption. The obtained values for the three metals were arranged in the sequence of: nickel > cadmium > cobalt (Akar *et al*., 2009).

3.5. Column Study of Initial Concentration

3.5.1. The Effect of Immobilized Biomass

Using initial or prepared biomass to adsorb heavy metals on a commercial scale has disadvantages such as the small size of low density particles, poor mechanical power and strength as well as solid and liquid phase isolation. Immobilization of biomass within a desired network can overcome these problems. On the other hand, it makes it easier to reuse the biomass "retrieval feature" (Sudha and Abraham, 2003; Yahaya *et al*., 2009). In this study, the *S. cerevisiae* biomass prepared with ethanol was immobilized with polysulfone and was used in continuous system studies. The results showed that the use of immobilized biomass will lead to an increase in the removal percentage by about 48% compared to mobile ethanol-treated biomass under the same conditions. This increase can be, to some extent, attributed to the presence of polysulfone which has an average adsorption removal of 2.83% for nickel, 3.05% for cobalt and 7.12% for cadmium metal ions. The highest uptake capacity of polysulfone was obtained at 0.019 mg g^{-1} for nickel, 0.020 mg g^{-1} for cobalt and 0.032 mg g^{-1} for cadmium. Similar studies were conducted with *S. cerevisiae* using different immobilizers, which determined immobilization benefits adsorption regardless of matrix material (Akar *et al*., 2009; Sudha and Abraham, 2003; Al-Saraj *et al*., 1999; Cabuk *et al*., 2007).

3.6. The Effect of Bed Height and Flow Rate on Biosorption

Metal adsorption in the packed bead column system highly depends on the value of the adsorbent within the column (Zhang *et al*., 2011). In the present study, the effect of the bead column height on the adsorption value of the yeast was studied for each metal at three heights of 20, 25 and 30 cm with varying biomass concentrations (i.e., 7, 9 and 11 g) for each sample. By increasing the bead column height, the removal percentage improved for cadmium, nickel and cobalt. With the highest removal percentage recorded at the bead height of 30 cm. **Figure 5** shows the breakthrough curves at the different heights of the bead column at which the parameters of pH 8, initial ternary metal ions concentration of 100 mg L^{-1} and the input flow rate of 1 mL min^{-1} were kept constant during the experiments. In addition, the results showed that an increase in the height of the bead column, from 20 to 30 cm will lead to an increase in the adsorption rate, the exhaustion time (t_e), breakthrough time (t_b) and the refined volume (Hasan and Srivastava, 2009; Zhang *et al*., 2011; Charumathi and Das, 2012). The curve slope will decrease with increasing bead height due to the increase of the available binding sites for adsorption and the increase of the mass transfer zone (ΔT) (Vijayaraghavan *et al*., 2005; Hasan and Srivastava, 2009). However, by increasing the bead column height at the constant initial metal ion concentration and flow rate, the MTZ length increased for all studied metal ions. In a bed bead with a higher length, a larger volume of the metals ion solution could be treated and a higher percent of metal ions removed, so that when the bed height increased 3 times (from 10 to 30 cm) the total percentage removal was by 51.01% for Cd(II), 42.18% for Ni(II) and 38.04% for Co(II). The maximum obtained adsorption capacity followed the order of Cd(II) > Ni(II) > Co(II).

The input flow rate is one of the important features in the evaluation of adsorbent in the adsorption of heavy metals for a wastewater continuous system, especially for industrial scaling. In this study, the varying effects from the input flow rate of 0.5, 1, 2 and 4 mL min^{-1} in the column height of 25 cm, pH 8 and the initial concentration of 100 mg L^{-1} of ternary metal ions of cadmium, nickel and cobalt on the amount of adsorption by *S. cerevisiae* was investigated (Figure not shown). The results showed that the removal percentage decreased by increase in the flow rate and can be attributed to the retention time of the medium in the column. With less time for the beads adsorbed the heavy metals in the medium was due to mass transfer inhibitions (Vijayaraghavan *et al*., 2005; Akar *et al*., 2009; Zhang *et al*., 2011; Charumathi and Das, 2012). The highest removal percentage was observed in the flow rate of 0.5 mL min^{-1}. The maximum adsorption of cadmium was observed followed by nickel and cobalt. The uptake capacity increased with an increase in the flow rate from 0.5 to 4 mL min^{-1}. In addition, the gradient of the breakthrough curve also increased as a result with the highest uptake capacity observed at a flow rate of 4 mL min^{-1} for cadmium and nickel and 2 mL min^{-1} for cobalt. Thus, the optimum flow rate must be selected with consideration to the mutual effects of the above mentioned factors (Vimala *et al*., 2011; Hasan and Srivastava, 2009; Hasan *et al*., 2010).

3.7. The Effect of Initial Ion Concentration on the Biosorption

The effect of ternary metal ions on the adsorption of three metals was investigated at four initial concentrations of 10, 30, 50 and 100 mg L^{-1} at pH 8, bead column height of 25 cm and a flow rate of 1 ml/min. Breakthrough curve is represented in **Fig. 6** as a function of time for the metal ions of cadmium at different concentrations. The results showed that time on the breakthrough curves will decrease with an increase in the initial concentration from 10 to 100 mg L^{-1}. This phenomenon can be attributed to the decrease in the length of the adsorption zone (Kiran and Kaushik, 2008) and to the fact that by increasing the initial concentration, bonding sites will be saturated faster (Zhang *et al*., 2011). The adsorption percentage was decreased with an increase in the initial metal ion concentration. The removal percentages (R) were 60.85, 55.50, 52.21 and 50.91% for 10, 30, 50 and 100 mg L^{-1} cadmium concentration, respectively. The reason can be attributed to the reduction or saturation of the adsorption sites in exchange for the increase in metal ions (Vieira *et al*., 2008). The uptake capacity will increase with the initial metal ion concentration. The uptake capacities (q_e) were 0.88, 1.94, 2.26 and 4.53 mg g^{-1} for cadmium for 10, 30, 50 and 100 mg L^{-1} cadmium concentration, respectively. However, as the influent concentration increases twice (from 10 to 100 mg L^{-1}), it provides a high concentration difference and the MTZ length increases to 17.3, 17.9, 21.5 and 22.8 cm for 10, 30, 50 and 100 mg L^{-1}, respectively and the breakthrough curve will be rather steep resulting in an earlier t_b and t_e, as shown in **Fig. 6**. These results suggested that the biosorption process can be applied to the treatment of dilute effluents.

3.8. Adsorption Modeling in Continuous System

To successfully design a continuous adsorption process, we need to model a breakthrough curve for the outgoing waste water. Thomas and Yan (1988) models were used to determine the maximum uptake capacity of beads in a continuous system.

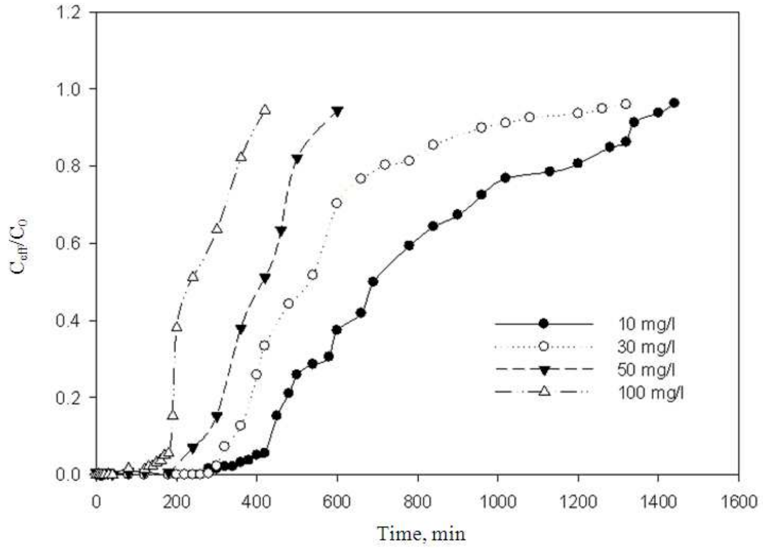

Fig. 6. Breakthrough curve at the different initial cadmium ion concentration and at constant flow rate 1 mL min^{-1}, height of the bead column 25 cm and pH 8

Table 2. Thomas and Yan (1988) models parameters every metal ion studied

Metal ions	Breakthrough analysis				Thomas model			Yan model		
	Q, ml/min	q_e, mg/g	R, %	MTZ, cm	k_{th} x 10^3, $L \cdot mg^{-1} \cdot h^{-1}$	q_{th}, mg/g	R^2	q_y, mg/g	a, min	R^2
Cd(II)	0.5	2.43	53.30	17.68	6.3	2.36	0.9931	2.29	4.0	0.9953
	1.0	3.74	51.05	21.97	7.4	3.67	0.9957	3.53	3.7	0.9973
	2.0	5.65	47.12	22.55	7.5	5.68	0.9801	5.00	2.4	0.9811
	4.0	7.12	45.76	22.14	13.3	6.40	0.9553	5.74	1.9	0.9891
Ni(II)	0.5	1.04	45.75	17.70	50.6	1.03	0.9960	1.01	5.0	0.9976
	1.0	1.57	42.18	19.10	39.5	1.58	0.9943	1.53	4.9	0.9910
	2.0	1.78	37.19	16.30	22.0	1.80	0.9924	1.76	4.9	0.9912
	4.0	1.96	33.97	21.90	18.7	1.97	0.9856	1.87	3.5	0.9760
Co(II)	0.5	1.19	45.23	18.33	13.0	1.18	0.9875	1.14	4.4	0.9914
	1.0	1.56	38.04	19.44	17.4	1.50	0.9777	1.43	3.8	0.9936
	2.0	2.03	30.97	20.51	21.3	1.94	0.9751	1.75	2.7	0.9925
	4.0	2.58	35.49	22.50	41.1	2.54	0.9524	2.37	1.9	0.9472

Table 3. Adsorption and desorption data of Ni(II), C0(II) and Cd(II)

	Ni(II)			Co(II)			Cd(II)		
Regeneration	q_e, mg/g	R, %	R_E, %	q_e, mg/g	R, %	R_E, %	q_e, mg/g	R, %	R_E, %
Cycle #1	1.59	44.02	94.77	1.47	40.80	90.69	3.70	49.35	92.39
Cycle #2	1.37	42.51	91.88	1.37	36.14	88.65	3.47	46.29	87.39
Cycle #3	1.16	39.80	89.16	1.24	34.44	81.77	3.35	43.09	86.35

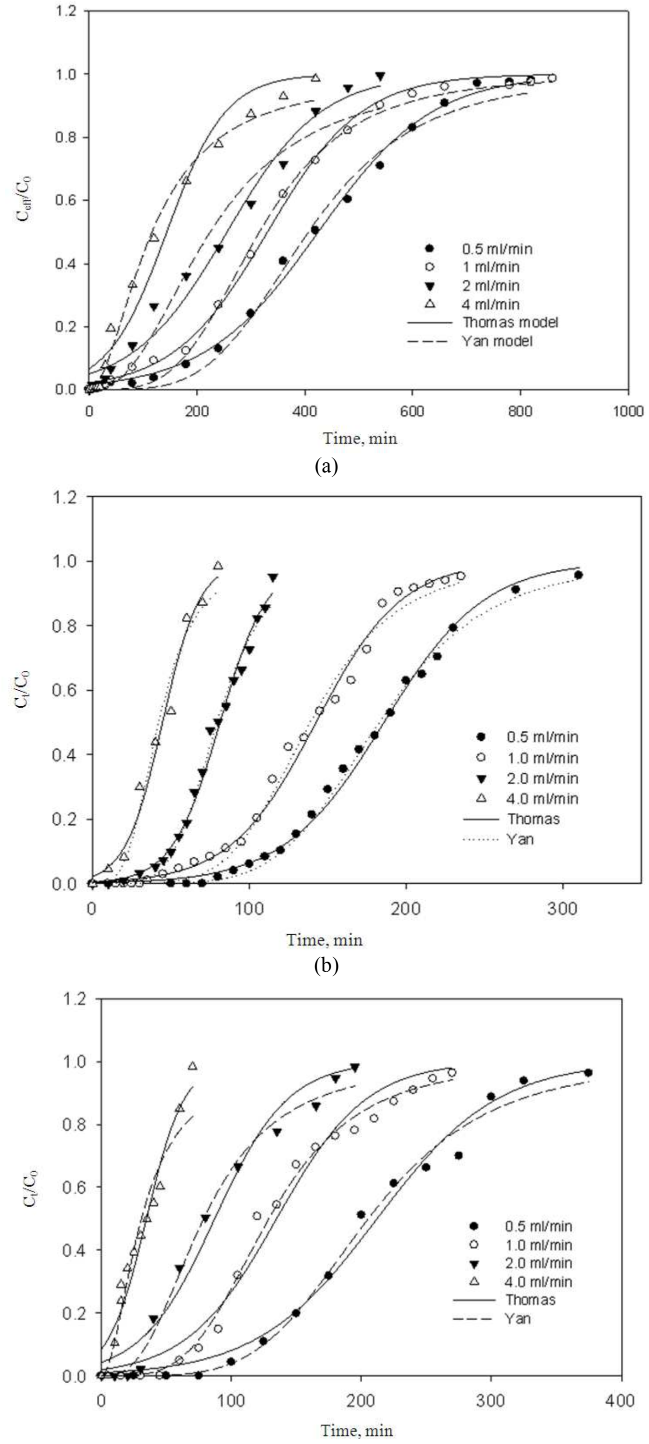

Fig. 7. Thomas and Yan (1988) models for Cd(II), Ni(II) and Co(II) metals ion at various flow rate and at constant flow rate 1 mL min^{-1}, height of the bead column 25 cm and pH 8

For this purpose, a column with bead height of 25 cm was used with the initial metal ion concentration of 100 mg L^{-1}, pH 8 and the varying flow rate of 0.5, 1, 2 and 4 mL min^{-1} was applied. Thomas and Yan (1988) models for cadmium, nickel and cobalt are shown in **Fig. 7**, which is based on the process of changing the concentration ratio between the treated volumes to the input waste over time. The statistical analysis showed that the concentration-time curves is well consistent with Thomas model (based on the probability less than 0.050 and the value of R^2) for all three metals. The value of q_{th}, K_{th}, q_y in these models was calculated based on the breakthrough curve data. In the present study, another prediction model that has been used is the breakthrough curve analysis through MATLAB software. The uptake capacities of biomass (q_{th} and q_y) and the constant predicted by Thomas model (k_{th}) are presented in **Table 2**. This model better predicts the output concentration at zero time, overcome the problems found in the Thomas model and also provides a better description of the breakthrough curve (Pokhrel and Viraraghavan, 2008). Yan model constants for the three studied metals are also shown in **Table 2**. One of the advantages of Yan modeling in comparison to using the Thomas model is the prediction of the output concentration for a fixed bed column over time. Thomas and Yan (1988) models correlated well to the test data (rang from 0.9524 to 0.9960 for Thomas and rang from 0.9472 to 0.9973 for Yan), but the difference between the experimental adsorption capacity (q_e) of cadmium, nickel and cobalt and the value predicted from the Thomas model (q_{th}) was considerably lower than the predicted value of the Yan model (q_y). This means that the Yan model overestimated the q_y value for all studied metal ions.

3.9. Regeneration

Biosorbent regeneration is one of the key factors in determining their potential for commercial applications. Reusing an adsorbent several times can reduce the costs. To investigate this effect, a column with 9g of biomass at a bead height of 25 cm was compressed. The flow rate of 1 mL min^{-1} was also applied to perform the subsequent biosorption cycles. In addition, 250 ml of the 1M HNO_3 solution was used in each regeneration cycle in order to regenerate the adsorbent. Three cycles of adsorption and desorption were performed to examine the reusability and metal recovery efficiency of the biosorbent and the results are shown in **Table 3**. As shown in **Table 3**, the Ni(II), Co(II) and Cd(II) adsorption capacities of the immobilized biomass decreased from 1.59 to 1.16 mg g^{-1}, 1.47 to 1.24 mg g^{-1} and 3.70 to 3.35 mg g^{-1} within three cycles, respectively. The recovery efficiency (R_E) also decreased from 94.77-89.16% for Ni(II), 90.69-81.77% for Co(II) and 92.39- 86.35% for Cd(II) within three cycles (**Table 2**). The removal of immobilized biomass.

For Ni(II), Co(II) and Cd(II) did not change significantly and only a maximum about 10% for Co(II) decrease was observed after three cycles.

4. CONCLUSION

In this study, the ternary metal ions of cadmium, nickel and cobalt have been successfully removed from aqueous solution using *S. cerevisiae* pre-treated with ethanol and immobilized with polysulfone in both batch and continuous systems. The optimal batch system conditions were achieved at pH 8, 8 g L^{-1} of biomass and the initial metal ion concentration of 100 mg L^{-1}. Under these conditions, the maximum uptake capacity of 3.1 mg g^{-1} was obtained for cadmium, 1.2 mg mg^{-1} for nickel and 0.68 mg mg^{-1} for cobalt. The optimal results obtained for the batch system were used in the continuous system. Optimal conditions in the continuous system were pH 8, bead column height of 25 cm, the approximate flow rate of 1 mL min^{-1} and the initial metal ion concentration of 100 mg L^{-1}. The calculated uptake capacity of ternary metal ions was 3.74 mg g^{-1} for cadmium, 1.57 mg g^{-1} for nickel and 1.56 mg g^{-1} for cobalt. Immobilization of *S. cerevisiae* by polysulfone provided 48% more heavy metal elimination in comparison non-immobilized ethanol-treated conditions.

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