Heavy Metals Biosorption in Liquid Solid Fluidized Bed by Immobilized Consortia in Alginate beads

R Ilamathi¹*, G S Nirmala², L Muruganandam³

¹Department of Biotechnology, SNIST, Yammampet, Hyderabad, Andrapradesh, India.
²³Chemical Engineering Division, School of Mechanical and Building Sciences, Vellore Institute of Technology, Vellore, India.

*Corres. author: mathidmagnate@gmail.com
Tel: +91-9014339148 Fax: 91-040-27640394

Abstract: Our work aims to throw light on biosorption of heavy metals in a Liquid Solid Fluidized Bed as a successful alternative for heavy metal removal. The design and fabrication of LSFB has been discussed. Batch studies and fluidized bed studies were carried out to study the biosorption behavior for chromium, nickel, copper and cadmium by alginate beads containing a mixed consortium of Yeast, Pseudomonas aeruginosa, Bacillus subtilis and Escherichia coli. Fluidized bed studies were carried out in 1m length and 5cm diameter column, with an optimized adsorbent dosage of 1g/L, a flowrate of 132 LPH, a bed height of length of the reactor. Efficiency of biosorption for copper, cadmium, chromium and nickel in LSFB was found to be 84.62%, 67.17%, 49.25% and 61.02%. The efficiencies were found to depend on the pH, temperature, initial metal concentration, and the residence time of the beads in the fluidized beds. Desorption of the exhausted beads was successful, however, with a reduced biosorption capacity. Pretreatment of the culture was found to increase the capacity of metal uptake.

Keywords: Liquid Solid Fluidized bed; Immobilization; Heavy metals; Biosorption; Desorption.

Introduction

One of the most challenging environmental problems is the removal of heavy metals and other toxic contaminants from industrial wastewater. Of the important metals, Mercury, lead, cadmium, Arsenic and Chromium (VI) are regarded as toxic; whereas, others such as copper, nickel, cobalt and zinc are not as toxic, but their extensive usage and increasing levels in the environment are of serious concerns [1,2,3]. Several methods are being used for the removal of heavy metals ions from aqueous wastes (Chemical Precipitation, Ion Exchange, Electrochemical Treatment, Membrane Technologies, adsorption on activated Carbon. etc. [4]. Each of these methods has its own merits and demerits. But the search for new eco-friendly and cost-effective technology for the removal of heavy metals from wastewaters has been directed towards biosorption.
Biosorption using potential metal biosorbents like algae, bacteria, fungi, and yeast can be an effective technique to decrease the concentration of heavy metal ions in solution [5]. Reduction of hexavalent chromium Cr(VI) to Cr(III) by bacteria such as Pseudomonas aeruginosa [6], Bacillus sp. [7] and Escherichia coli [8] is already reported. However, application of free bacterial cells at industrial scale is disadvantageous due to the difficulty of biomass/effluent separation [9] etc., which may be overcome by using immobilized bacterial cells with the advantages of stability, regeneration, solid–liquid separation and minimal clogging in continuous systems [10]. Immobilization of microorganisms in a suitable matrix like polyvinyl alcohol, agar media and sol–gel materials has been proven to be an efficient solution to this problem [11,12].

Adsorption processes are traditionally carried out in fixed beds [13] due to the high concentration of solids and the obtainable uniform residence time. However since the wastewater to be treated often contains solid impurities leading to a plugging of the fixed bed, the liquid must be clear to avoid column blocking. Recently, many experimental studies have been conducted in fluidized beds, which allow treatment of turbid liquids while avoiding the channeling problems [14]. Fluidized beds are common and important reactors in process engineering because of the good mass and heat transfer rate between the fluid and the particles, and between the particles and the side wall of the column. The term fluidization is used to describe the condition of fully suspended particles. Liquids or gases are passed at certain velocity up through a bed of solid particles, at this velocity the pressure drop across the bed counter balances the force of gravity on the particles and further increase in velocity achieve fluidization at a minimum fluidization velocity. Fluidization quality is closely related to the intrinsic properties of particles, e.g. particle density, particle size and size distribution, and also their surface characteristics [15]. From the previous literature related to the biosorption of heavy metal using bacteria, it can be concluded that there is a lack in literature of using immobilized bacterial consortia in liquid solid fluidized bed to study the behavior of biosorbents and its efficiency in heavy metal adsorption. Hence, this work aims to study the adsorption of heavy metals like chromium, nickel, copper and cadmium in liquid solid fluidized bed using immobilized sol-gels as a solid catalyst containing mixed cultures of Yeast, Pseudomonas aeruginosa, Bacillus subtilis and Escherichia coli.

Materials And Methods

2.1 Materials

2.1.1 Microorganisms

Pseudomonas aeruginosa, Bacillus subtilis, E.coli, Yeast obtained from the laboratory culture collection was maintained in the specific medium and appropriate proportions used for the experiment. Standard sterile techniques were used for inoculation of cultures. Medium used for the microorganism and all the glassware were properly sterilized autoclaved at 15 lb/in² pressure and 121°C for 30 minutes.

2.2 Methods

2.2.1 Preparation of Metal Solutions

Different metal concentrations were prepared by dissolving of CuCl₂, CdCl₂, NiSO₄ and K₂Cr₂O₇ salts in double distilled water in equal ratio to have metal concentrations of 50, 100, 150, 200, 250 and 300 mg/L. A stock solution of 1000mg/L was prepared all other concentrations are obtained from it. All glassware washed with 0.1 M HCl before and after each experiment to avoid binding of the metal to it.

2.2.2 Preparation of biosorbent

The culture was transferred and grown on specific media (Bromifield medium-Bacillus subtilis; Cetrimide medium-Pseudomonas aeruginosa; YPD-Yeast; LB medium-E.coli) for subculture. 100 ml of sterilized culture media was transferred to 250 ml Erlenmeyer flask. The media was allowed to cool and then the 100µl microbial solution was inoculated into the medium in laminar air flow chamber. The inoculated flasks were incubated in an orbital shaker at 250 rpm at 32°C for 2 days to obtain the biomass. Mixed cultures were prepared by adding equal amounts of individual cultures. Biomass was harvested from the medium by centrifugation at 9000 rpm for 10 min. The supernatant was discarded and the cells were re-suspended in double distilled water (MilliQ)
for washing and again centrifuged as above to make sure that no media remain on the cell surface. This biomass was used for sorption studies.

2.2.3 Immobilization of biosorbent

A 3% (w/v) solution of sodium alginate was prepared by thoroughly mixing the alginate beads in hot distilled water with continuous stirring. To the Alginate solution, 10 ml of fresh culture is added and mixed properly. Care has to be taken that there is no lump formation. The mixture of Sodium Alginate and culture is then poured into the burette. The mixture was extruded using a burette into 0.5 M CaCl$_2$·2H$_2$O. The resultant beads were allowed to stay in the Calcium Chloride solution for 2 hours for hardening, following which, the Calcium Chloride is drained and the beads are washed in distilled water, kept in the freezer. The beads were kept at 4°C for 12 h, and thawed at room temperature for further use.

2.2.3 Batch biosorption studies

2.2.3.1 Optimization of parameters

Batch biosorption studies were carried for the determination of various parameters such as pH, time, temperature, initial metal concentration and bed height. Biosorption experiments were conducted at an initial metal concentration of 100 mg/L and 100 mg sorbent in 100 ml of metal solution at 30°C for 3 hours at pH varying from 1.0 to 7.0 by adding 0.01 N HCl. The effect of temperature on sorption was determined at 10, 20, 30, 40 and 50°C. Effect of contact time was studied at an initial metal concentration of 100 mg/L and 100 mg sorbent in 100 ml solution at 30°C and at optimized pH and temperature. Samples were analyzed for the concentration of metal at regular interval of one hour for 24 hours. Further biosorption studies at optimized conditions were also carried out with initial metal concentrations in the range of 50–300 mg/L of metal solutions prepared as stated in section 2.2.1. Biosorbent dosage was optimized at different amounts of biosorbent as 25, 50, 75, 100, 125 and 150 mg in 100 ml of 100 mg/L of metals solutions. The optimized condition for the biosorption studies of mixed consortium were analyzed.

2.2.4 Experimental Protocol for LSFB

2.2.4.1 Design and Fabrication of a LSFB

Liquid Solid Fluidized Bed (LSFB) was designed for the continuous biosorption of heavy metals. A 100cm length and 5cm diameter column was used. During adsorption metal solution from the tank was circulated using pump. The pH is measured with an on-line pH meter. For a fluidized bed, the liquid flowrate should be above the minimum fluidization velocity in order for fluidization to take place. However, it should not exceed the terminal velocity of the particle, causing subsequent particle depletion in the bed. The minimum fluidization velocity ($U_{mf}$) and the terminal velocity ($U_t$) was calculated using the equation 1 and 2 and it was found to be 42.965 LPH and 546.419 LPH respectively.

$$U_{mf} = Re_{mf} \mu_f / \rho_f d_p$$

$$U_t = \left( 4(\rho_p - \rho_f) g^2 / 225 \mu_f \rho_f \right)^{1/3} d_p$$

2.2.4.2 Design of distributor plate

The distributor has been designed with an opening area of 6.5% of the total area of the distributor plate. The diameter of the distributor plate was 5cm, which was same as that of the diameter of the column. The pores have been arranged in a triangular pitch. The diameter of the hole was designed based on the particle size to entrap the immobilized beads inside the LSFB reactor.

2.2.4.3 Experimental setup

Fig. 1 shows the fabricated LSFB consists of a homogenizing section 14 cm long for the uniform mixing of the inlet wastewater before it enters the reactor. Above the homogenizing section is the distributor plate of diameter 5 cm, with pores of diameter 1.5mm arranged in triangular pitch. Above the distributor plate is an acrylic riser of 88 cm, which functions as the LSFB. There is a solid disengaging section above the riser, from where the liquid effluent is withdrawn. Pressure tapings were made at different heights. Ball valves are used for the inlet and the bypass to have a controlled flowrate of metal solutions inside LSFB. The LSFB requires a ½ HP pump.
for the inlet. A rotameter of range 0 – 300 LPH was used to vary the flowrate. Immobilized Sodium Alginate beads were selected as the fluidizing particle. The main advantage of using Sodium Alginate is that it doesn’t react with water. Also, it has small diameter and low density, and hence, it is easier for fluidization and entrainment. Another advantage is that it fluidizes at low liquid flowrate. Hydrodynamics studies were carried out on the LSFB using Alginate beads as the fluidizing particle to study its behavior and to check its functioning.

2.2.4.4 Selection of fluidizing particle

The distributor pore diameter is 1.5mm. A particle of size smaller than 1.5mm will pass through the distributor or plug the distributor. Hence, the particle size should be greater than 1.5mm. Synthetic waste water was the fluid being used, the fluidizing particle has to have density greater than that of used waste water, failing which, the particles will float and fluidization will not occur. Hence the density of the particle must be greater than 1000kg/m^3. Hence, Sodium Alginate beads were selected as the fluidizing particle. The main advantage of using Sodium Alginate is that it doesn’t react with water. Also, it has small diameter and low density, and hence, it is easier for fluidization and entrainment. Another advantage is that it fluidizes at low liquid flowrate.

2.2.4.5 Experimental Procedure

The fluidized bed is initially filled with beads up to a 1/4th of the total riser volume. Tap water is pumped from the reservoir into the reactor column using a ½ HP pump. The flowrate of the liquid is measured using a rotameter with a range of 0 to 300 LPH. At each flowrate, the bed height is measured and tabulated. The pressure drop across the column is also measured using a digital manometer and tabulated. The voidage is calculated at minimum fluidization velocity and at different flowrates. The pressure drop across the bed was found to be the same for different flowrates, thus indicating the proper construction of the LSFB.

2.2.4.6 Hydrodynamics of LSFB

Hydrodynamics studies were carried out on the LSFB using Alginate beads as the fluidizing particle to study its behavior and to check its functioning. The effect of flowrate was analyzed with pressure drop, bed height and voidage to ensure the proper construction and fabrication of Liquid Solid Fluidized Bed.
2.2.5 Biosorption studies in LSFB

Immobilized biosorbent prepared as per section 2.2.3 was filled in the fluidized bed till 1/4th of the riser volume. 30 liters of synthetic heavy metal solution was prepared. The prepared heavy metal solution (100mg/L) was pumped through the column at desired flow rate of 132 LPH which was determined by the hydrodynamic studies in the column. Optimized parameters were used, and the heavy metal concentration was determined using atomic absorption spectrophotometer at 5 min interval in the start of the experiment and then at 15 min intervals subsequently from the sampling port. The fluidized bed studies were carried out at pH 4.5 at room temperature. The effect of pressure drop and bed height on different flowrate on heavy metal adsorption was studied. Samples were collected at pre-defined time intervals, centrifuged as above and the amount of metal in the supernatant was determined.

2.2.6 Determination of metal concentration in the supernatant

The heavy metal concentration was determined by the use of atomic absorption spectrophotometer, VARIAN 3600. Determination of copper, chromium, cadmium and nickel was done by using its specific lamp for each metal and at a specific wavelength.

2.2.6.1 Data evaluation

The amount of metal bound by the biosorbents was calculated using equation 3.

\[ Q = \frac{v(C_i - C_f)}{m} \]  

(3)

Where \( Q \) is the metal uptake (mg metal per g biosorbent), \( v \) the liquid sample volume (ml), \( C_i \) the initial concentration of the metal in the solution (mg/L), \( C_f \) the final (equilibrium) concentration of the metal in the solution (mg/L) and \( m \) the amount of the added immobilized biosorbent on the dry basis (mg).

2.2.7 Desorption studies

The exhausted Alginate beads containing immobilized microorganisms after heavy metal biosorption were removed from the LSFB. The beads were then treated with 0.1 N Nitric Acid, and allowed to stay for an hour and loaded back into the LSFB. 10 ml of samples were withdrawn every half hour. The samples were then analyzed in the Atomic Absorption Spectrophotometer to determine the heavy metal concentration.

2.2.8 Pretreatment Studies

For pretreatment, 0.1 N NaOH was prepared and poured into the mixed culture in the ratio 1:2. The mixture of culture and alkali were placed on the shaker incubator at 150 rpm and 35°C. After an hour, the culture is mixed with Sodium Alginate and immobilized beads are made. These beads form the pretreated beads. The beads are then loaded in the LSFB. Synthetic wastewater is prepared by making 30 liters of 70 ppm heavy metal solution in the feed tank. The pump is switched on and the flowrate is set to an optimum of 132 LPH as determined by the hydrodynamic studies in the column. 10 ml of samples are withdrawn every half hour. The samples are then analyzed in the Atomic Absorption Spectrophotometer to determine the heavy metal concentration.

3 Results and Discussion

3.1 Batch sorption studies

3.1.1 Effect of batch sorption parameters

3.1.1.1 Effect of pH

The experimental results of chromium, cadmium, nickel and copper using mixed cultures of Yeast, Pseudomonas aeruginosa, Bacillus subtilis and Escherichia coli at varying pH was shown in the Fig. 2(A). Effect of pH on biosorption has been studied over a range of 1 to 7. The highest removal of cadmium and copper was found at pH 4, while at pH 3 and 5 the highest removal of chromium and nickel obtained respectively. At pH<3, lowest biosorption was recorded and the experiments beyond pH 5 were hindered due to precipitation of metals during the biosorption process.
Fig. 2 Batch Studies. Effect of (A) pH. (B) Contact time (C) Temperature (D) Initial Metal Concentration (E) Biosorbent Concentration of the immobilized bacterial consortium.
3.1.1.2 Effect of contact time

Before the starting of the batch experiments, it was very important to know the required time to reach equilibrium biosorption. In other words, for all batch experiments, the contact time should be sufficient to ensure reaching an equilibrium concentration. Fig. 2 (B) shows the results of biosorption removal of heavy metals concentration versus contact time for the four metal solutions. It can be seen that a 3 hour contact time is sufficient to reach equilibrium for all four heavy metals.

3.1.1.3 Effect of temperature

Biosorption studies of Metals solutions using immobilized mixed cultures were carried out at different temperatures ranging from 10°C to 50°C. The effect of temperature on metal sorption was presented in Fig. 2(C). The percentage of metal sorption was increased from 10°C to 30°C and then showed decrease in sorption percentage with increase in temperature. Fig. 2(C) shows a maximum percent of sorption around 90% achieved at 30°C at fixed pH 4.5 and biomass concentrations 2.0 mg/ml. This decrease in biosorption efficiency at temperatures 40-50°C may be attributed to: an increase in the relative desorption of the heavy metals from the solid phase to the liquid phase, deactivation of the biosorbent surface, destruction of active sites on the biosorbent surface due to bond disruption, or due to the weakness of the sorbent active site binding forces and the sorbate species and also between the adjacent molecules of the sorbed phase. From Fig. 2(C) it was concluded that experiments can be carried out at room temperature.

3.1.1.4 Effect of initial metal concentration

Fig. 2 (D) shows the variation of percentage sorption of immobilized microbial culture at different initial metal concentrations. The results indicated that the percentage biosorption of metals was not altered greatly from concentrations between 50-150 mg/L whereas still increasing in the initial metal concentration beyond 150 mg/L shows a decrease in the sorption capacity, this may be due to the sorbent dosage depicted in Fig. 2 (E) which was found to be 1 g/L of immobilized biosorbent containing enough biosorption surfaces and sites for this concentration.

3.2 LSFB studies for biosorption

3.2.1 Hydrodynamics Studies of LSFB

3.2.1.1 Pressure drop Vs flowrate

From Fig. 3(A), it is evident that the pressure drop across the LSFB increases continuously till the flowrate of the liquid attains that of the minimum fluidization velocity, which is the velocity required to fluidize the particles, in this case, the Alginate beads. On further increase of the flowrate of the liquid beyond minimum fluidization velocity (43 LPH), the pressure drop across the LSFB is found to remain nearly constant, hence obtaining a straight line in the latter part of the graph. This signifies that once the fluidization starts, the pressure drop across the LSFB remains the same for different flowrate.

3.2.1.2 Bed height Vs flowrate

Fig. 3(B) shows that the bed height of the constructed Liquid Solid Fluidized Bed (LSFB) remains constant till the flowrate reaches the minimum fluidization velocity, which is 43 LPH for the above mentioned bed. Since the beads are supposed to fill 25% of the entire volume of the reactor, the static bed height is 22 cm. Once the flowrate of the liquid exceeds the minimum fluidization velocity, the fluidization of the beads starts and the bed expands. The bed height was found to increase more or less linearly with the liquid flowrate. This indicates the proper construction of the LSFB. The optimum flowrate was determined as 132 LPH since it was found that at this flowrate, the bed height is the length of the reactor. In other words, at a liquid flowrate of 132 LPH, the beads filled the entire length of the column, without any overflow.
3.2.1.3 Voidage Vs Flowrate

For the constructed LSFB, Fig. 3(C) bed voidage was found to remain more or less constant till the liquid flowrate attained the minimum fluidization velocity, which is 43 LPH. Once the liquid flowrate exceeded the minimum fluidization velocity, the voidage of the bed increased, which indicates that the particles got fluidized. It was found that the voidage increased with the increase in the liquid inlet flowrate.
3.2.2 LSFB biosorption studies

Biosorption studies in LSFB were conducted, which was packed with the known quantity of immobilized alginate beads. The synthetic metals solution was pumped at optimized flowrate of 132LPH. The metal concentrations were determined by atomic absorption spectrophotometer at an interval of 15 min from the sampling port. Fig. 3(D) shows the plot of metals concentration in the LSFB against the residence time in the fluidized bed. As observed from the batch studies, maximum amount of metal is absorbed in the first 90 minutes for cadmium. Fig. 3(E) shows the biosorption efficiency in LSFB to the residence time. The maximum efficiency of biosorption of Cadmium by immobilized beads in the LSFB was found to be 67.17%. Biosorption of Chromium in the LSFB was also found to increase up to 90 minutes, after which the biosorbed metal leached back into the solution. The maximum biosorption efficiency for uptake of Chromium (VI) metal was calculated to be 49.25%. The optimum time for the biosorption of nickel was determined to be around nearly 3 hours, after which the biosorption efficiency remains more or less constant. The maximum biosorption efficiency was determined to be 62.02%. Similar to the batch studies performed for Copper, the concentration of Copper in the solution was found to continuously decrease in the first 3 hours. The maximum biosorption efficiency of Cu in the LSFB was determined to be 84.62%. In order to increase the efficiency, higher residence time will be required. Biosorption of copper in the LSFB were found to be high than other metals this may be due to the high suitability of optimized parameters and enough surfaces and sites for this concentration. Fig.3(C) gives the maximum biosorption efficiency of metals in which the copper biosorption efficiency in the LSFB was determined to be high as 84.62%. Biosorption efficiency of the other metals may increase by optimizing the
biomass concentration of each microorganism with respect to the metals solution before immobilizing in alginate beads.

3.3 Desorption studies

The desorption of exhausted Alginate beads containing mixed culture using 0.1N Nitric Acid. Fig. 4(A) shows the concentration was found to decrease from 40 ppm to 27.12 ppm for copper. Fig. 4(B) represents the biosorption efficiency of copper. The efficiency was found to decrease from 82.4% to 33.46%.

3.4 Pretreatment Studies

Fig. 4(C) shows for the Biosorption of Copper using immobilized beads containing mixed consortium pretreated with 0.1N NaOH. Fig. 4(D) was found that on pretreatment, the biosorption capacity of the microorganisms for copper was increased from 84.62% to 86.54%.

4. Conclusion

Cadmium, Chromium, Copper and Nickel are not only toxic heavy metals but also carcinogenic. Hence, it becomes imperative to search for cost-effective and efficient method for continuous removal of these contaminants. The present study evaluated the removal of copper, chromium, nickel and cadmium from synthetic metals solutions using immobilized consortium of Yeast, *Pseudomonas aeruginosa*, *Bacillus subtilis* and *Escherichia coli* in alginate beads in fluidized bed reactor. The design and construction of an LSFB was done. Hydrodynamic studies were performed on the fabricated LSFB, and the design was ensured to be done correctly. Batch experiments showed that the mixed culture immobilized in alginate beads can be successfully used for the removal of these metal ions. LSFB studies showed that the removal of copper and nickel was high compared to chromium and cadmium. This concluded that biosorption was found to depend significantly on pH of the solution. Optimum flowrate 132 LPH obtained for LSFB results in good contact time between the adsorbent and adsorbate. Desorption and pretreatment were also done for the metal copper and found to be effective. It can thus be concluded from this project work that biosorption of heavy metals in an LSFB is not only a cost effective method, but also a highly efficient alternative to chemical and physical treatments employed for the removal of heavy metal pollutants from contaminated water. Further work will be carried out in developing a model with plastic beads instead of sodium alginate with different biomass concentration. Realtime application of the process may be studied by using wastewater from an industry rather than synthetic wastewater.

References

1. B. Brown, M. Absanullah, Effects of heavy metals on mortality and growth, *Mar Pollut Bull.* 2(1971) 182–187.
2. J. W. Moore, Inorganic Contaminants of Surface Water Residuals and Monitoring Priorities, New York: Springer-Verlag, 1990 pp. 178–210.
3. B. Volesky(ed), Biosorption of heavy metals. CRC Press, Boca Raton, Florida, 1990.
4. J. T. Matheickal, Q. Yu, Biosorption of lead (II) and Copper (II) from aqueous solution by pretreated biomass of Australian marine algae. *Bioresour Technol.* 69(1999) 223-229.
5. B. Volesky, Biosorbent materials, *Biotechnol Bioeng Symp.* 16(1986) 121-126.
6. H.X. Song, Y.G. Liu, W.H. Xu, G.M. Zeng, N. Aibibu, L. Xu, B.B. Chen, Simultaneous Cr(VI) reduction and phenol degradation in pure cultures of *Pseudomonas aeruginosa* CCTCCAB91095. *Bioresour. Technol.* 100 (2009) 5079–5084.
7. Y. Wang, C. Xiao. Factors affecting hexavalent chromium reduction in pure cultures of bacteria. *Water Res.* 24 (1995) 2467–2474.
8. G.F. Liu, H. Yang, J. Wang, R.F. Jin, J.T. Zhou, and H. Lv, Enhanced chromate reduction by resting Escherichia coli cells in the presence of quinone redox mediators. *Bioresour. Technol.* 101(2010) 8127–8131.

9. C. White, S.C. Wilkinson, G.M. Gadd, The role of microorganisms in biosorption of toxic metals and radionuclides. *Int Biodeterior Biodegradation.* 35(1995) 17–40

10. M. Viera, G. Curutchet, E. Donat, A combined bacterial process for the reduction and immobilization of chromium. *Int Biodeterior Biodegrad.* 52(2003) 31–34

11. M.A. Saraj, M.S. Abdel-Latif, I. El-Nahal, R. Baraka, Bioaccumulation of some hazardous metals by sol–gel entrapped microorganisms. *J. Non-Cryst. Solids.* 248 (1999) 137–140.

12. J. Xu, X.C. Song, Q. Zhang, H. Pana, Y. Liang, X.W. Fan, Y.Z. Li, Characterization of metal removal of immobilized Bacillus strain CR-7 biomass from aqueous solutions. *J. Hazard. Mater.* 187(2011) 450–458.

13. G. J. McDougall, C. A. Fleming, Extraction of precious metals on activated carbon, in Ion Exchange and Sorption Processes in Hydrometallurgy, Ed. by Streat, M. and Naden, D., *Critical Reports on Applied Chemistry.,* 19 (1987) 56-126.

14. Y. Fu, D. Liu, Novel experimental phenomena of fine-particle fluidized bed. *Experimental Thermal and Fluid Science.* 32 (2007) 341-344

15. J.F. Richardson, J.H Harker, J.R. Bachurst, Coulson and Richardson’s CHEMICAL ENGINEERING, Particle Technology and Separation Processes”, Vol.(2), 5th Ed, Butterworth-Heinemann, 2002.

*****