THE MECHANISM OF CHROMIUM BIOSORPTION BY SACCHAROMYCES CEREVISIAE

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Abstract The Biosorption property of S. cerevisiae for chromium uptake was investigated in an immobilized cell bioreactor. Saw dust was utilized as the solid bed in the reactor. Adsorption of S. cerevisiae on saw dust obeys a first order reaction kinetic up to 6 hours. The immobilized biomass particles are porous and exist in the new generation of biological adsorbent. Chromium biosorption was studied in this bioreactor. The maximum uptake capacity of the heavy metal is a function of the initial chromium concentration. Chromium biosorption obeys a Freundlich isotherm kinetic model.

Key Words Biosorption, S. Cerevisiae, Chromium Uptake, Biological Adsorbent, First Order Reaction Kinetic, Freundlich Isotherm

چکیده خصوصیت جذب بیولوژیکی کروم توسط میکروارگانسیم ساکارومیسس سرویسیه در یک راکتور بستر ثابت بررسی شد. از ذرات چوب بعنوان حامل در این راکتور استفاده گردید. جذب میکروارگانیسم ساکارومیسس بر روی ذرات چوب تا ۶ ساعت از سینتیک درجه اول متابعت می نماید. ذرات سلول تثبیت شده در ردیف اول مواد بیولوژیکی جاذب قرار دارند. میزان جذب حداکثر کروم تابع غلظت اولیه یون فلزی است. جذب کروم توسط میکروارگانیسم با مدل سینیتیکی فرندلیچ مطابقت دارد.

INTRODUCTION

Since some heavy metals are known for their ecological toxic contamination, a great attention has been paid to the study of heavy metal accumulation by microorganisms, both from the view points of metal recovery and removal from the aquatic environment. The term biosorption has been used to describe a property of biomass which retains ions of heavy metal, radionucleotides or valuable metals. The cell wall of a live or dead microorganisms acts as a basic building block for the accumulating of ions [1]. Alemzadeh et al [2] observed that flocculation of bacteria with the aid of cations like Ca^{+2} or Al^{+3} is the results of ionic bond bridge formed between the negatively charged cell surface and the cation in the solution. Cell walls of procaryotes and eukaryotes contain polysaccharides, with ion exchange properties [1].

Strandgerg et al. [3] demonstrated that the uranium-complexing capacity of extracellular polysaccharide isolated from yeast cells of S. cerevisiae were directly related to the content of the individual polymers. Penda et al. [4] reported the possibility of using Aspergillus niger in removing hexavalent chromium from water. The effect of parameters as age of pellets, pH, and ions were studied on biosorption properties of A. niger. High levels of radionucleotide as uranium and thorium from aqueous solution have been identified by Tsezos and Voleskey [1,5] with non living biomass of Rhizopus arrhizus. Heavy metal pollution has become a topic of general public concerns. As reported by Culp G. and Culp R. [6], heavy metals have been classified as having very high or high pollution potential. Very high pollution potential metals include Ag, Au, Cu, Hg, Pb, Sb, Sn,

Te, and Zn, and high pollution potential metals include: Ba, Bi, Fe, Mn, Mo, Ti and U. The disposal of industrial wastewater into the rivers, streams and lakes disturbs the ecosystem of these water bodies. The effluents like electroplating, chloro-alkali, leather industries and the drainage of mines cause metal pollution. Many investigators including Aimal et al. [7]; Gould and Genetelli [8]; Rudo et al. [9]; Abernathy et al. [10] Newman et al.[11] Mouvet et al. [12,13] offered different methods for different heavy metal removal. In this investigation chromium Cr^{+6} Biosorption from a synthetic waste was studied by S. cerevisiae yeast cell immobilized on saw dust particles.

MATERIALS AND METHODS

Microorganism: Saccharomyces cerevisiae ATCC 4126. The strain was grown in shaking culture or sabourd's liquid medium in a 500 ml Erlenmeyer at 30°C. After 48 h, the cells were harvested by Heraeus Christ II KS centrifuge (10 min at 5000*g) and were washed once with distilled water.

Preparation of immobilized cell bioreactor was made according to the method described by Alemzadeh [14]. For this reason, the yeast cells were suspended in 200 ml of physiological serum, 0.9 g sodium chloride /100 ml distilled water. The reactor was made of plexiglass, with 20 cm long, 2.5 cm diameter, reactor total volume, 98 ml. The reactor was filled with 3 g saw dust as the support, the void fraction reached about 0.25. A peristaltic pump, Cole Parmer WZIPO51, was used to circulated the cell suspension through the reactor at a flow rate of 170 ml/h at room temperature (Figure 1). The cell adsorption was determined by the dry weight technique using the millipore 0.45 micrometers filter paper. The amount of adsorbed cell was

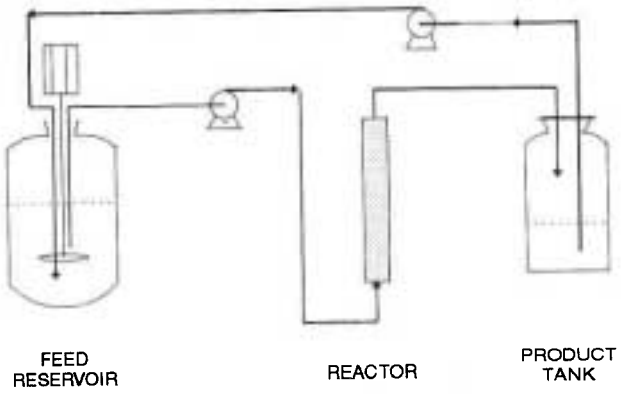

Figure 1. Schematic Re presentation of the Bioreactor

calculated by the difference between initial cell concentration and concentration after certain time and it is defined as adsorption capacity, mg cell/g support [15].

The chromium (Cr^{+6}) solution was prepared according to the standard method reported by Greenbery [16]. The chromium concentration was determined using pye Unicam SP 191 Atomic Absorption Spectrometer (ATS). The synthetic chromium waste was diluted, and after circulating the reactor at flow rate of 50 ml/h, the residual chromium concentration was measured by the same ATS method. A blank system with only saw dust (no microorganism) was carried out. The chromium solution 100, and 1000 ppm was separately circulated into the reactor. The reduced chromium concentration C/C_0, was derived where, C: chromium concentration at a certain time and C_0: Initial chromium concentration. The maximum chromium (biosorbent) uptake value (q) was determined when biosorption reaches the steady state condition and it is defined as mg chromium /g, Immobilized cell.

RESULTS AND DISCUSSION

The immobilization behavior of S. cerevisiae on saw dust is shown in Figure 2. As the figure presents cell

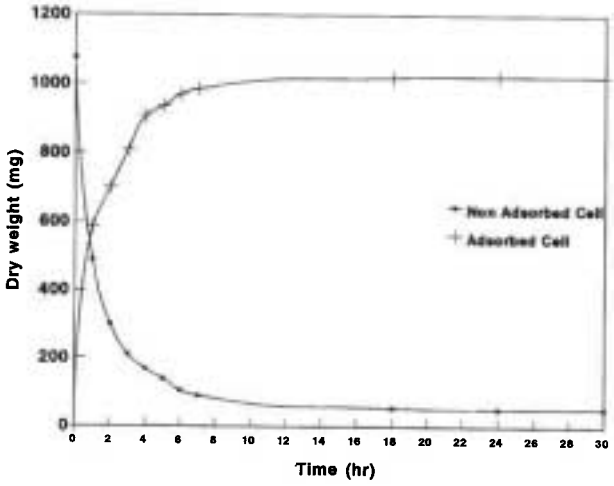

Figure 2. Concentration of adsorbed or non adsorbed (free) cell with time.

adsorption is linear up to 6 hours and then reaches a steady state condition. The amount of final cell adsorption (adsorption capacity) was 420 mg/g of support. The adsorption capacity of S. cerevisiae immobilized on wood chips in ethanol production was 248 mg/g, [15]. These authors also reported that yeast cells have high adsorption capacity comparing to other types of microorganisms. Gerson and Zajic [17] demonstrated that yeast cells also show significant adhesive strength.

A logarithmic plot of free cell concentration (cell in solution) mg, against time is shown on Figure 3 which is linear. Therefore, the adsorption velocity obeys first order reaction kinetic which is in accordane with langmuir isotherm model (Equation 1).

$$\text{Ln } C/C_0 = -Kt \qquad (1)$$

The constant K from Figure 3 was determined: $K = 0.343h^{-1}$.

Immobilization of Saccharomyces yeast cell on corn stover in invert sugar production was also as langmuir isotherm model [14].

Chromium biosorption was also studied in the immobilized cell bioreactor. Different chromium base solutions were circulated into the bioreactor. The maximum biosorbent uptake value (q) was calculated for different initial chromium concentrations. The reduced chromium concentration ratio with time is shown in Figure 4. Along with the results obtained for blank system carried out with only saw dust and chromium circulation at 100 and 1000 ppm, the

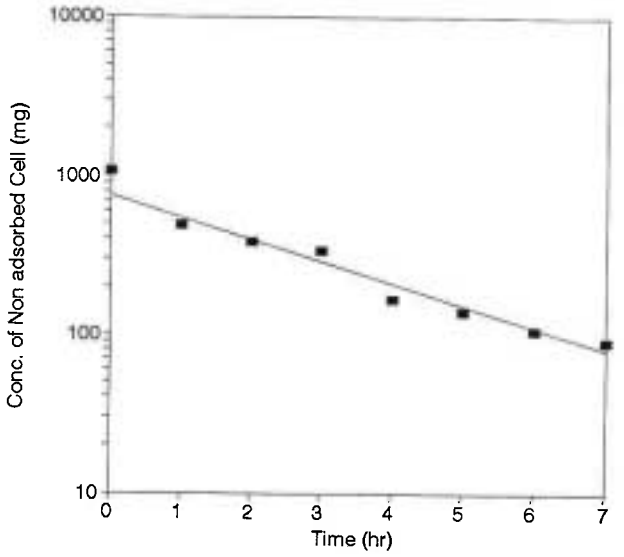

Figure 3. Change of non adsorbed cell (free cell, cell in solution) concentration with time.

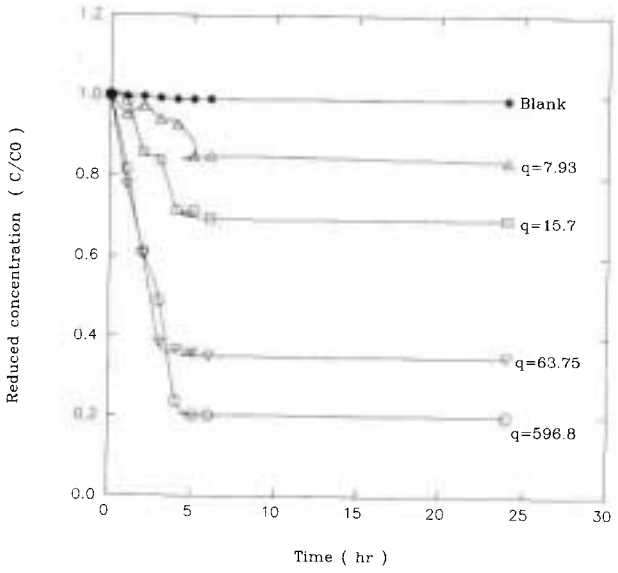

Figure 4. Reduced chromium concentration ratio with time for blank and different maximum uptake value (q).

reduced concentration ratio with time is also presented in Figure 4. Each point in the blank curve is the average of two different concentration ratios. In the case of blank, one can conclude that the capacity of saw dust in retaining chromium is negligible. The results presented in Figure 4 also demonstrate that the velocity of chromium biosorption at the maximum amount of chromium concentration (1000 ppm) is significant that is, biosorption rate increases with the initial chromium concentration. The maximum chromium uptake value was 596.8 mg/g dry cell mass, which belongs to the highest initial chromium concentration (1000 ppm).

Table 1 presents the effect of initial chromium concentration on the maximum % of chromium biosorption.

The biosorption percent is a function of the initial chromium concentration. For the initial chromium concentration of 1000 ppm, a maximum percent of biosorption (about 80%) was observed. Strandberg et al. [3] demonstrated that yeast cells of S. cerevisiae can accumulate heavy metals as uranium for an amount of 150 mg/g cell weight.

Schumate and strandberg [19] reported that pure culture of a microorganism exhibits heavy metal uptake ranging from 8 to 35% dry weight. Retaining 65-68 mg of heavy metal (copper and cadmium)/g protein was reported by chang et al. [20].

For different initial chromium concentrations (C), the amount of maximum uptake capacity (q, mg Cr^{+6} /g cell) was determined. A plot of *ln*q vs *ln*C yields a

TABLE 1. Effect of Initial Chromium Concentration on Maximum % of Chromium Biosorption.

Initial Cr^{+6} concentration (ppm)	% of Cr^{+6} biosorption
42.5	16.3
50	30.7
100	65.0
1000	79.9

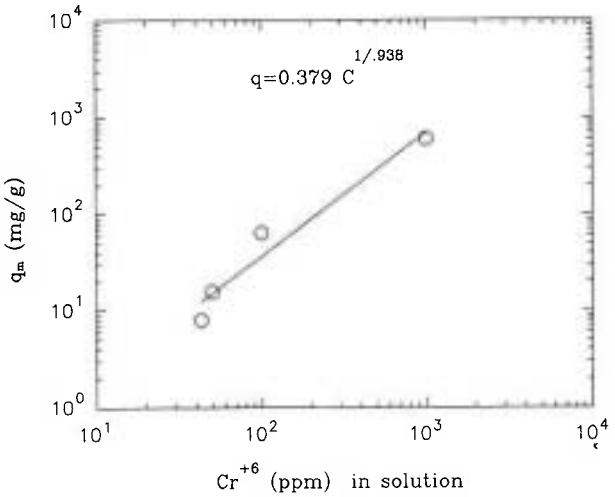

Figure 5. Linearized chromium biosorption isotherm by S. Cerevisiae.

straight line with *ln* K as the intercept and 1/n as the slope. The results are shown in Figure 5. The higher initial chromium concentration results in a significant biosorption value. The chromium biosorption model fits the Freundlich isotherm (Equations 2,3). Tsezos and Voleskey [1] observed the same properties for radioactive metal adsorption by microorganisms.

$$q = KC^{1/n} \quad (2)$$

$$\ln q = \ln K + 1/n \ln C \quad (3)$$

K and n are empirical constants: K = 0.379, n = 0.938

CONCLUSION

A batch scale reactor was operated under laboratory conditions to study the yeast cell adsorption on saw dust as the solid support and the behavior of cell adsorption. Saccharomyces were utilized as biosorbent due to its high sorption capacity. The final amount of adsorbed cell on saw dust was 420 mg/g support, which is significant. The cell adsorption on the solid support obeys the langmuir isotherm model. The chromium biosorption was studied in the reactor

with time. For different initial chromium concentrations , the heavy metal biosorption was measured. About 80% of chromium was biosorbed by the reactor. The effect of the initial chromium concentration on the maximum uptake value was calculated, which was 596.8 mg Cr^{+6}/g cell for 1000 ppm the initial chromium concentration. The logarithm of the heavy metal biosorption vs the initial chromium concentration is linear which obeys a Freundlich adsorption isotherm model.

ACKNOWLEDGEMENT

The authors are pleased to thank the Research vice chancellor in Sharif University of Technology.

REFERENCES

1. M. Tsezos and B. Voleskey, "Biosorption of Uranium and Thorium", *Biotechnol. Bioeng.* 23, (1981), 583-604.
2. I. Alemzadeh, Y. Maeda and A. Fazeli,. Bacterial Flocculation with Na-Bent. *J. Ferment. Technol.* 55, (1977), 181-188.
3. G.W. Strandberg, S. E. Shumate and Jr. Parrott, "Microbial Cells as Biosorbents for Heavy Metal Accumulation of Uranium by S. Cerevisiae and Pseudomonas aeroginosae", *Appl. Evironment. Microbiol.* 41, (1981), 237-245.
4. T. Penda, R. Sureshkumar, C. Krishna, Prasad, N. S. B. N. Venkaraman, S. Staishkumar and S. Anand Venkatesh, "Biosorption of Hexavalent Chromium by Aspergillus Niger Pellet", Achema 94, Frankfurt Germany, Environmental Technology, (1994).
5. M. Tsezos and B. Voleskey, "The Mechanism of Uranium Biosorption by R. Arrhizus", *Biotechnol. Bioeng.* 24, (1982), 385-401.
6. G. Culp and R. Culp, "Heavy Metal Removal In: New Concepts in Water Purification", Van Nostrand Reinhold Company, New York, (1974), 218-227.
7. M. Aimal and A. U. Khan, "Effect of Water Hardness on the Toxicity of Cadmium to Microorganisms", *Wat. Res.*, 18 (12) (1984), 1487-1491.
8. M. Gould and E. Genetelli, "Heavy Metal Complexation Behavior in Anaerobically Digested Sludges," *Wat. Res.* , 12, (1978), 505-512.
9. T. Rudo, R. M. Sterritt and J. N. Lester,. "Formation and Conditional Stability Constants of Complex Formed between Heavy Metals and Bacterial Extracellular Polymers," *Wat. Res.*, 18, (3), (1984), 379-384.
10. A. R. Abernathy, G.,L. Larson and R. C. Mathews,. "Heavy Metals in the Surficial Sediments of Fontana Lake," North Carolina, *wat. Res.* 18 (3), (1984), 351-354.
11. M. C. Newman, A. W. McIntosh and V. A. Greenhut,. "Geochemical Factors Complicating the Use of Aufwuchs as a Biomonitor for Lead Levels in Two New Jersey Reservoirs," *Wat. Res.*, 17 (6), (1983), 625-630.
12. C. Mouvet and A.C.M. Boung, "Speciation (Including Adsorbed Species) of Copper Lead, Nickel and Zinc in the Meuse river," *Wat. Res.* 17, (6). (1983), 641-649.
13. M. Tsezos, S. Noh, and M. H. I. Baird,." A Batch Reactor Mass Transfer Kinetic Model for Immobilized Biomass Biosorption," *Biotechnol. Bioeng.* 32, (1987), 545-553.
14. I. Alemzadeh,. "Immobilization de l'invertase Sur Rafle de Mais. Comparaison Avec des Cellule Immobilisees." These, No 16 Toulouse France, (1981).
15. J. Navarro and G. Durannd,. "Modification of Yeast Metabolism by Immobilization onto Porous Glass," *Eur. J. Microbiol.* 4, (1977), 243-254.
16. A. E. Greenbery,. "Determination of Antimony,... by Direct Aspiration into an Air-acetylene Flame, Atomic Absorption." In: Standard Methods for the Examination of Water and Wastewater, 6th ed. American Public Health Assorciation, (1985),157-160.
17. D. F. Gerson and J. E. Zajic,. "Biophysics of Cellular Adhesion ACS Symp," Ser, 106, (1979), 29-57.
18. I. Alemzadeh and M. Vossoughi,."Surcrose Inversion

by Saccharomyces Yeast Celis Immobilized onto Corn Grits," *J. Eng. (IRI)*. 2 (3/4) (1989) 84-87.

19. S. E. I. Shumate and G.W. Strandberg,. Accumulation of Metals by Microbial Cells, "In Comprehensive Biotechnology," M. Moo-Young et al. (Eds). Pergamon Press New York, Ny, Vol. 4, (1985) 235-247.
20. D. Chang, K. Fukushi and S. Ghosh, . "Simulation of Activated Sludge Cultures for Enhanced Heavy Metal Removal, *Water Environment. Research*, 67, (1995), 822-827.