



Removal of nickel and total chromium using *Escherichia coli* biofilm supported on clinoptilolite

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Original Article

Abstract

Biofilm is communities of microorganisms attached to the surface and is able to concentrate metal species within their cell structure. Therefore, the aim of this study was to produce *Escherichia coli* biofilm on zeolite (clinoptilolite) and evaluate its ability for nickel (Ni) and chromium (Cr) adsorption from aqueous solutions. A laboratory-scale batch model was used for biosorption assay. The effect of initial metal concentrations and pH on the removal efficiency was studied. Two isotherm equations were used for analyzing the experimental data. The results showed that Ni uptake by biofilm were higher than Cr. The biosorption process was best described by the Langmuir model. Fourier transform infrared confirmed that there are some functional groups on the biomass surface that may interact with the metal ions. It is concluded that the biofilm is very promising for the removal of metal ions from aqueous solution and hence may be encourage the utilization of biofilm in environmental applications.

KEYWORDS: Nickel, Chromium, Biofilm, Biosorption, Zeolite, *Escherichia Coli*

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Introduction

Environmental pollution by heavy metals has led to serious problems for humans and other organisms in the environment due to their nonbiodegradability and accumulation in the living organisms.¹⁻³ Conventional methods for removing heavy metals from industrial effluents contains various processes such as precipitation, coagulation, ion exchange, electro dialysis, electrocoagulation, reverse osmosis, evaporation and filtration.^{4,5} However, most of the above mentioned processes have significant economic and technical limitations such as low efficiency, high sludge production, disposal of sludges containing large amounts of heavy metals, need

for specific chemicals and expensive regeneration process.^{4,6} Therefore, it is recommended to find new alternatives and biosorption can be considered as a new method.³

Biosorption is a process in which formation of usually chemical bonds between metal ions and functional groups on the surface of some biomaterials; such as bacteria, fungi, algae and agricultural residues; leads to separation of metals from aqueous solution. Biosorption mechanism is usually related to the chemical bonding between the adsorbent functional groups (metabolically mediation uptake) and the metal ions or ion-exchange reaction due to the high ion exchange capacity of the biosorbent (physicochemical adsorption). Economical nature and an ecofriendly behavior are the major advantages of a biosorption. Hence, is

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being developed as an alternative to conventional methods for the removal of heavy metals. However, high affinity for metal uptake, rapid uptake and maximum adsorption capacity are some of the significant factors that should be considered in biosorbent selection. Most microorganisms such as bacteria, fungi, yeasts and algae are able to concentrate metals from aqueous solutions and to accumulate in their cell structure.⁷ Among the various biosorbents, bacteria because of their small size, ubiquity, growing under controlled conditions and adaptation to a broad range of the environmental situation are used as an efficient biosorbents.^{5,7} Bacteria can produce macromolecules, known as extracellular polymeric substances (EPSs), which is important in the development of biofilms.^{5,8} ESP is mainly composed of polysaccharides, nucleic acids, proteins, lipids or humic substances which contain several functional groups such as the carboxyl, amine, hydroxyl and phosphoric groups.^{5,9} Among various functions of ESP in biosorption processes, the most important ones are adhesion to cell surfaces and accumulation of elements from the environment.^{9,10} However, batch adsorption of pollutants and possible exiting from the reactor are two major problems of biofilm that reduce efficiency and cause operational problems.^{3,6,9} Therefore, a supported media for biofilm can be useful to reduce the above problems.

Several synthetic and natural materials have been used as supporting materials for bacterial biofilms. Among the various media, materials with a porous structure and high adsorption affinity to pollutants and biofilm would be a suitable alternative. Since zeolite has ion-exchange properties, thus it can be used as a supporting media for the bacterial biofilms. Zeolite is a mineral with a crystalline structure and contains numerous microscopic pores.¹¹ Ion-exchange properties of zeolite are the main reason for metals uptake. However, other mechanisms such as pore structure and surface charge are also involved in metals uptake.¹²

Based on the zeolites chemical structure, and the amount of exchangeable cations in their chemical structure, zeolites can show different capacities for metals uptake.^{12,13} As other researchers have pointed out, raw zeolite is considered as an available and low cost material with different capacity for heavy metals' uptake.^{11,13} Wingenfelder showed raw zeolite has a high capacity to absorb lead but, its ability to adsorb cadmium is less.¹⁴ Adsorption capacity of zeolite can be increased by modification of their chemical structure or surface properties. A new method for this purpose is surface coverage of zeolite by a specific biofilm by which not only continuous treatment is applicable by biofilm, but also keeps it within the reactor.^{3,6,7} For example, Quintelas et al. used *Escherichia coli* biofilm on granular activated carbon for the adsorption of hexavalent chromium (Cr) from industrial wastewater.³ This study aimed to investigate the ability of a *E. coli* biofilm, an effective agent for metal adsorption, supported on zeolite (clinoptilolite) for the removal of nickel (Ni) and Cr from aqueous solutions.

Materials and Methods

The bacterium *E. coli* (PTCC 1330) was obtained from Iranian Research Organization for Science and Technology. Heavy metals stock solutions were prepared by diluting $K_2Cr_2O_7$ and $NiCl_2(6H_2O)$ in distilled water. Atomic absorption spectrometric standards were prepared from 1000 mg/l metal solutions. The clinoptilolite (a natural zeolite) was obtained from Afrandtooska Co. The chemical compositions of clinoptilolite are given in table 1.

Medium for growth of *E. coli* was prepared using 5 g/l beef extract, 10 g/l peptone, 5 g/l NaCl at pH 7.2. The prepared medium was sterilized for 20 min at 121°C, cooled to room temperature, inoculated with bacteria and kept at 37°C for 24 h with moderate stirring in incubator. Batch adsorption experiments were conducted using 2 g of clinoptilolite with 15 ml of *E. coli* culture media and without *E. coli* (for comparison purposes) and 100 ml of the

different concentration of Ni and Cr solutions varied between 50 mg/l and 1000 mg/l in 250 mL beaker. The beakers were stirred at a constant rate of 120 rpm at 37°C for about 10 days (time required to reach an equilibrium, accordingly to previous studies).^{1,3} Then, 1 ml samples were taken, centrifuged and analyzed for metal ions concentrations. The determination of metal ions concentrations was performed by atomic absorption spectrometry (PG-990, England). The Fourier transform infrared (FT-IR) spectra of biosorbents were taken in KBr pellets using a Tensor 27 spectrophotometer (Bruker Optik GmbH, Germany).

Table 1. Chemical composition of clinoptilolite

Component	Weight (%)
SiO ₂	66.50
Al ₂ O ₃	11.80
TiO ₂	0.20
Fe ₂ O ₃	1.30
CaO	3.10
MgO	0.70
K ₂ O	3.00
Na ₂ O	2.00
P ₂ O ₅	0.01
L.O.I.	12.00

Biosorption capacity was determined from the difference between the initial and residual concentrations of the metal ion in the solution at the sampling time as follows:¹⁵

$$Q_t = \frac{V(C_i - C_t)}{m}$$

where Q_t is the amount of metal adsorbed per unit mass of biosorbent at a given time (mg/g), V the volume of solution (L), C_i and C_t are the initial concentration and the residual concentration of metal ions at a given time (mg/l), and m is the dry weight of the biosorbent (g). In order to evaluate the biosorption capacity, the experimental data were fitted to Langmuir and Freundlich isotherm models.

Results and Discussion

The solution pH is one of the most important factors that affect biological sorption process, because of its effect on the charge state of metals and also adsorbent surface. Therefore, effect of pH on studied metals uptake by zeolite and biofilm was evaluated and shown in figure 1. As is known, the cationic metals' adsorption efficiency is lower at acidic pH. It was observed that Ni and Cr uptake increased with increase in pH up to pH of 6 and then it decreased with any further increase in pH. It is clear that at pH below 6, hydrogen ions compete with positive charge ions for adsorption sites. Therefore, this condition limits the access of Ni ions to the adsorption sites and decreases Ni removal.³ Furthermore, at alkaline pH values, the adsorption of Ni decreases because soluble hydroxylated complexes of the metal ions is formed and compete with Ni for adsorption sites.¹⁶ According to Bhattacharyya and Gupta, biological uptake of Ni decreases at pH values higher than 8 due to precipitation of Ni ion as a hydroxide.¹⁷ Based on other studies, the optimal pH for the biosorption of Ni was around 6 due to the effect of pH on metal speciation and metal binding sites on the biomass surface.¹⁸⁻²⁰

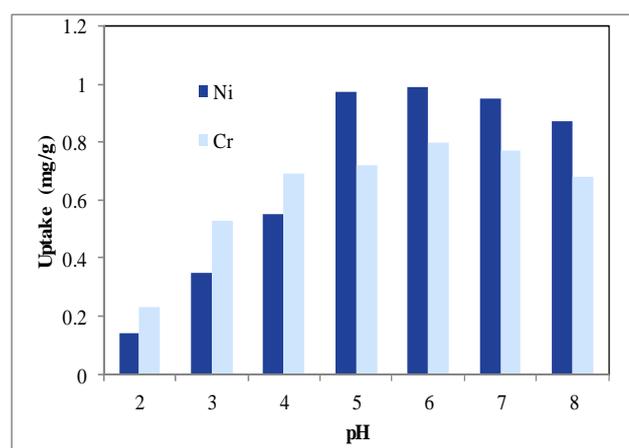


Figure 1. Effect of pH on the uptake of nickel and chromium by zeolite/biofilm (C₀ = 10 mg/l)

Cr adsorption changes trend at different pH

is similar to Ni. For pH values higher than 6, the formation of hydroxyl complexes of Cr and increasing the number of hydroxyl ions in the solution and its competition with CrO_4^{2-} for adsorption sites on the surface of the adsorbent, are the most reason for decreasing of Cr uptake.⁷ At pH lower than 6, Cr is in an anionic state and its biosorption decreases due to the competition with ions like nitrate ion from nitric acid that is used to adjust the pH and maintenance of low acidic condition.²⁰ Hence, in this study all adsorption experiments carried out in pH value of 6.

Figure 2 shows the effect of initial concentration of Cr and Ni on the removal of studied metals and their uptake. As is clear, the equilibrium adsorption capacity for the adsorption of Ni and Cr ions increased with increasing their initial concentration. In fact, in the solution with a high metal concentration, ions can be adsorbed better. While the removal efficiency showed the reverse trend. Hence, the metal removal rate decreased with increase in initial concentration. The maximum amount of Ni adsorbed per mass of adsorbent (zeolite and zeolite/biofilm) for concentrations of 10, 30, 50, 70, 90 and 110 mg/l were obtained 0.53, 1.26, 1.85, 2.45, 2.7 and 2.75 for zeolite and 1.01, 2.41, 3.96, 5.23, 6.21 and 7.46 for zeolite/biofilm, respectively. Instead, the removal percentage decreased from 53% to 25% for zeolite and from 88 to 59 for zeolite/biofilm with increasing of Ni concentration from 10 to 110 mg/l. Similarly, the maximum amount of Cr adsorbed per mass of adsorbent (zeolite and zeolite/biofilm) for concentrations of 10, 30, 50, 70, 90 and 110 mg/l were obtained 0.4, 1.14, 1.9, 2.1, 1.98 and 2 for zeolite and 0.8, 2, 2.81, 3.22, 3.2 and 3.3 for zeolite/biofilm, respectively. Instead, the removal percentage decreased from 40% to 18% for zeolite and from 70 to 26 for zeolite/biofilm with increasing of Cr concentration from 10 to 110 mg/l. These results are shown in figure 2.

In the adsorption process, the initial concentration of ions in solution has a key role as a driving force to overcome the mass transfer

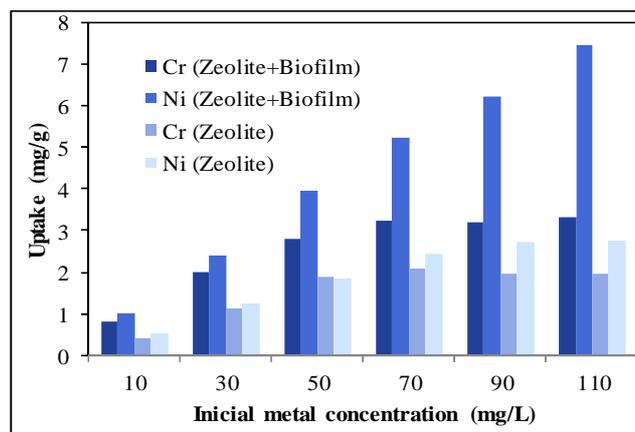


Figure 2. Effect of initial metal concentration on uptake values of nickel and chromium

resistance between the liquid phase and solid phase. Thus, it is expected that with an increase in metal ions the metal uptake will increase.²¹ The results showed that Ni and Cr uptake rate were relatively high at the beginning of the process due to abundance available adsorption sites. However, the number of active sites for the adsorption gradually decreases with an increase in process time and the adsorption rate slightly decreased, leading to the formation of the adsorption equilibrium. At low metal concentrations, adsorption sites are available and could easily be occupied since the ratio between the number of metal moles in solution and available surface area is low. Therefore, absorption is independent of the initial concentration. Nevertheless, at high concentrations of metal the removal efficiency is dependent on the initial concentration because the number of available sites is less than the number of metal moles. Horsfall et al. stated when the initial concentration increases, the removal rate decreases. At higher concentration, the number of ions competing for the available binding sites on the biomass surface increases and thus reduces the number of binding sites.²² Meanwhile, at higher concentration, the average distance between the adsorbed species will be reduced, which affects the distribution of surface charge. Thus, the ability of adsorbate to migrate to the adsorbent surface changes and, as a result,

the fixation reduces.⁷ Akhtar et al. showed that at high concentrations, the absorption is insignificant due to saturation of the binding sites of biosorbent.²³ However, increase in the biomass capacity at higher concentrations can be related to differences in metal ion concentration gradient between the solution and inside of the microbial mass.⁷ Finally, at very high concentrations of metal ions, solid-liquid equilibrium is limited by diffusion of metal ions into the microbial mass.⁷ In other words, the metal ions should diffuse through intraparticle diffusion into the biomass surface, and this mechanism is slow.²⁰ Also, comparison of the results of the adsorption of studied metal ions by zeolite with and without biofilm bacteria showed better results in the adsorption process when the bacterial biofilm is used, which confirms the important role of biofilms in biological uptake. In fact, zeolite provides necessary surface for biofilm development and then biofilm provides a high concentration of biomass per unit volume hence bacteria can remain inside the reactor for an unlimited period, resulting in increased electrostatic interactions between metal ions and extracellular polymers.⁷ Functional groups responsible for the bioadsorption process are mainly carboxyl, hydroxyl, carbonyl, sulfonate, amide, imidazole, phosphonate and phosphodiester groups. Most of these groups exist on *E. coli* biofilms, leading to their interaction with metal ions.²⁰ This fact has been confirmed by Pradhan et al.²⁴

Comparison of biosorption performance showed differences between Ni and Cr uptake. As Ni showed better results. Chemical properties of metals (valence, electronegativity, ionic radius and atomic weight) and properties of the biomass (structure, functional groups and surface area) cause the main differences in biosorption capacity.¹⁶ Accordingly, the electronegativity, ionic radius and atomic weight of Ni are 1.8 Pauling, 69pm and 58.7 u, respectively, and for Cr they are 1.6 Pauling, 44

pm and 51.99 u, respectively.^{16,20} Ni has a higher electronegativity than Cr and then better retentions on the biomass surface, which promotes the penetration into the EPSs.²⁰

Despite the lower ionic radius and atomic weight of Cr, which promotes Cr ions penetration into the polymeric net, low adsorption capacity of Cr is justified by its anionic state.²⁰ This unexpected result may also be related to xenobiotic effect caused by the Cr ions on the bacterium biofilm. Since metal binding to the bacterial biofilm is a metabolic pathway that is sensitive to the metal toxicity.²⁰ Barros et al. expressed the low removal of Cr by the zeolite is related to the difference between big anionic radius of Cr and the porous diameter of the zeolite and also the strong propensity of Cr to form complexes in the aqueous solution.²⁵

Adsorption isotherms are an important factor in system design and describe the interaction between the adsorbent and adsorbate. Two different models—Langmuir and Freundlich – were fitted, and sorption isotherm parameters calculated by linear regression (Figures 3 and 4) for the best fit and are shown in table 2.

When zeolite was used as the adsorbent, the best fit for Cr was obtained with the Langmuir isotherm ($R^2 = 0.95$) and for Ni the best fit was the obtained with the Freundlich isotherm ($R^2 = 0.98$). However, when zeolite/biofilm was used, the best fit for Ni and Cr was obtained with the Langmuir isotherm. Langmuir coefficients of determination (R^2) for Ni and Cr adsorption were obtained 0.98 and 0.95, respectively, which indicating a good fit of the Langmuir monolayer model for the uptake of Ni and implies a good mathematics adaptation. It may due to the homogeneous distribution of adsorption sites on the adsorbent surface. Langmuir isotherm assumes the adsorbent surface as homogeneous. However, Freundlich isotherm is important because it do not assume a homogeneous surface. According to the results, it is observed that the Freundlich model well describes the uptake of Ni

by zeolite. As shown in table 2, the value of Q_L for Ni is 3.96 mg/g, which is about 1.52 times larger than that for Cr, showing a higher biosorption capacity of the biosorbent for Ni

than that for Cr. A similar observation was reported by Quintelas et al.³ for higher biosorption capacity of the biosorbent for Ni.^{5,20}

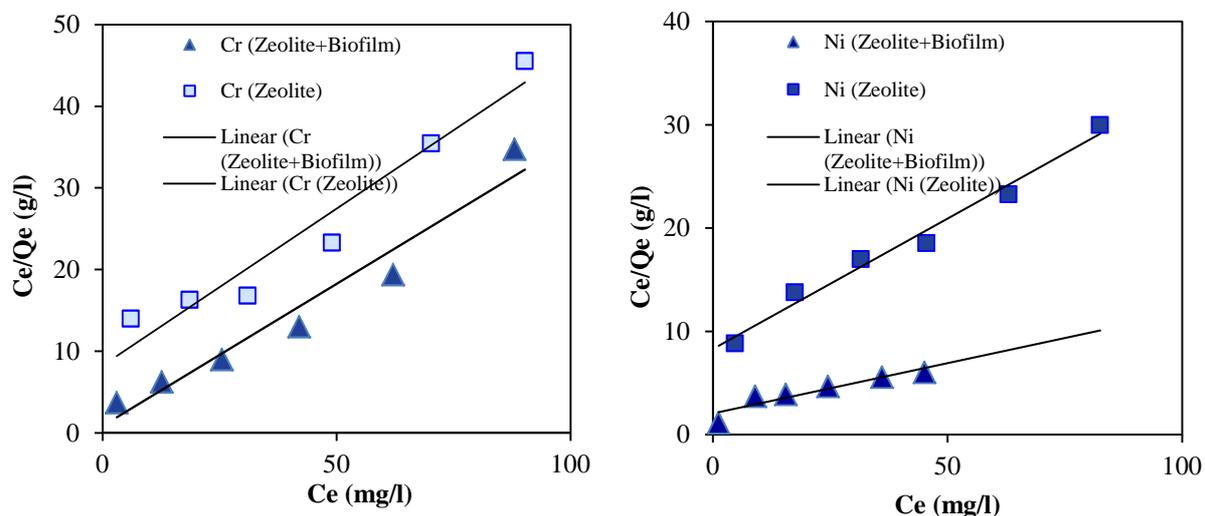


Figure 3. Biosorption isotherms of nickel and chromium based on the Langmuir model

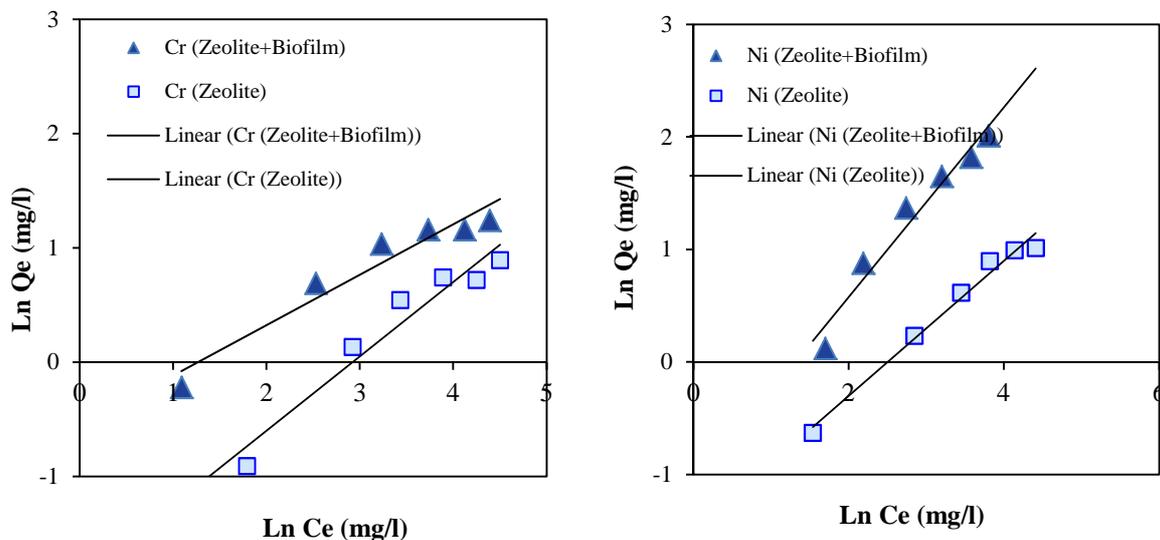


Figure 4. Biosorption isotherms of nickel and chromium based on the Freundlich model

Table 2. Parameters of Langmuir and Freundlich isotherms for biosorption of Ni and Cr

Type of adsorbent	Type of metal	Freundlich model			Langmuir model		
		K_F (mg/g)	b_F (l/g)	R^2	K_L (L/g)	Q_L (mg/g)	R^2
Zeolite	Ni	0.21	0.60	0.98	0.047	10.29	0.87
Zeolite + biofilm		0.33	0.84	0.96	0.031	3.96	0.98
Zeolite	Cr	0.15	0.65	0.94	0.400	2.87	0.95
Zeolite + biofilm		0.57	0.44	0.94	0.046	2.60	0.95

Ni: Nickel; Cr: Chromium; K_F : Freundlich constant; b_F : Degree of linearity between the solution concentration and the amount adsorbed; R^2 : Linear regression coefficient; K_L : Langmuir constant related to the affinity of binding sites; Q_L : Langmuir adsorption capacity

Figure 5 shows the FT-IR spectra of unloaded and metal loaded microbial biomass in the range of 1000-4000/cm. This spectrum was performed to determine the functional groups that might be responsible for the biosorption process. As the unloaded biomass spectrum shows, there are a number of absorption peaks, which reflecting the complex nature of the biomass. Based on the results obtained by Volesky²⁶ and Pavan et al.²⁷ hydroxyl, carbonyl, carboxyl, sulfonate, amide, imidazole, phosphonate and phosphodiester groups are main functional groups responsible for a biosorption process which some of them present on the *E. coli* biomass. Comparison between the spectra showed that unloaded biomass spectrum has a slight change in certain bands after uptake of metals. Therefore, these changes indicate a possible role of those functional groups in biosorption process.

Conclusion

This study was conducted to determine the ability of zeolite with and without *E. coli* biofilm for biosorption of Ni and Cr. It was found that the biosorption process is pH-dependent for both metals, and the maximum uptake was obtained in the pH of 6.

The equilibrium adsorption capacity for metal uptake increased with increasing initial concentration while the removal efficiency showed a reverse trend. The best isotherm fit for Ni and Cr was obtained with Langmuir model. FT-IR analyses showed that the main functional groups on the biomass were hydroxyl, carboxyl and phosphate groups that may be the main binding sites for biosorption of the Ni and Cr by *E. coli*. The presence of the biofilm increased the uptake efficiency of the zeolite that confirms the important role of biofilms in the adsorption process.

Conflict of Interests

Authors have no conflict of interests.

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References

1. Maleki A, Zarasvand MA. Heavy metals in selected edible vegetables and estimation of their daily intake in Sanandaj, Iran. *Southeast Asian J Trop Med Public Health* 2008; 39(2): 335-40.

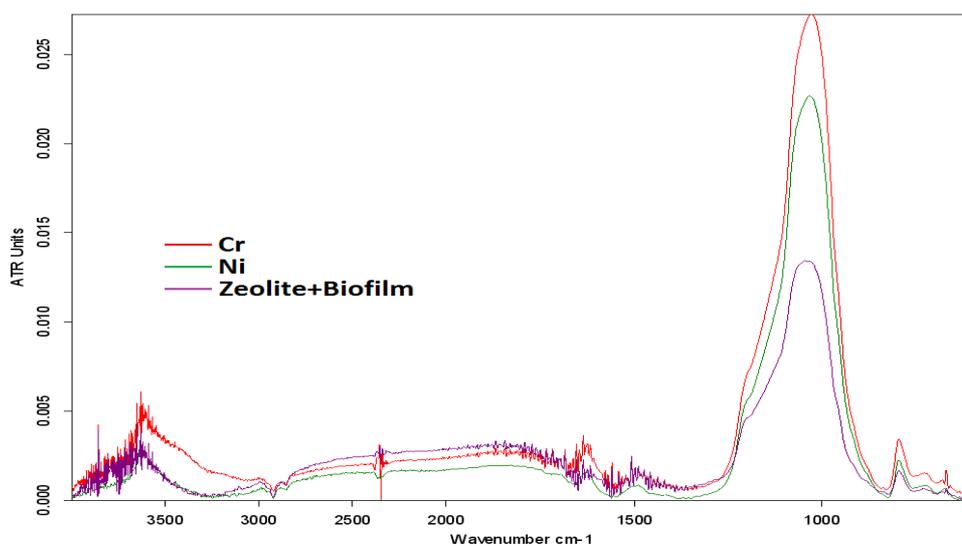


Figure 5. Fourier transform infrared spectra of zeolite/biofilm before and after metal loading

- Maleki A, Mahvi AH, Zazouli MA, Izanloo H, Barati AH. Aqueous Cadmium Removal by Adsorption on Barley Hull and Barley Hull Ash. *Asian J Chem* 2011; 23(3): 1373-6.
- Quintelas C, Rocha Z, Silva B, Fonseca B, Figueiredo H, Tavares T. Biosorptive performance of an *Escherichia coli* biofilm supported on zeolite NaY for the removal of Cr(VI), Cd(II), Fe(III) and Ni(II). *Chemical Engineering Journal* 2009; 152(1): 110-5.
- Maleki A, Erfan MB, Mohammadi AS, Ebrahimi R. Application of commercial powdered activated carbon for adsorption of carbolic acid in aqueous solution. *Pak J Biol Sci* 2007; 10(14): 2348-52.
- Quintelas C, Fernandes B, Castro J, Figueiredo H, Tavares T. Biosorption of Cr(VI) by a *Bacillus coagulans* biofilm supported on granular activated carbon (GAC). *Chemical Engineering Journal* 2008; 136: 195-203.
- Lameiras S, Quintelas C, Tavares T. Biosorption of Cr(VI) using a bacterial biofilm supported on granular activated carbon and on zeolite. *Bioresour Technol* 2008; 99(4): 801-6.
- Quintelas C, Fonseca B, Silva B, Figueiredo H, Tavares T. Treatment of chromium(VI) solutions in a pilot-scale bioreactor through a biofilm of *Arthrobacter viscosus* supported on GAC. *Bioresour Technol* 2009; 100(1): 220-6.
- Comte S, Guibaud G, Baudu M. Biosorption properties of extracellular polymeric substances (EPS) towards Cd, Cu and Pb for different pH values. *J Hazard Mater* 2008; 151(1): 185-93.
- Eboigbodin KE, Biggs CA. Characterization of the extracellular polymeric substances produced by *Escherichia coli* using infrared spectroscopic, proteomic, and aggregation studies. *Biomacromolecules* 2008; 9(2): 686-95.
- Wang W, Wang W, Zhang X, Wang D. Adsorption of p-chlorophenol by biofilm components. *Water Res* 2002; 36(3): 551-60.
- Leung S, Barrington S, Wan Y, Zhao X, El-Husseini B. Zeolite (clinoptilolite) as feed additive to reduce manure mineral content. *Bioresour Technol* 2007; 98(17): 3309-16.
- Kocaoba S, Orhan Y+, Akyuz T. Kinetics and equilibrium studies of heavy metal ions removal by use of natural zeolite. *Desalination* 2007; 214(1-3): 1-10.
- Castaldi P, Santona L, Enzo S, Melis P. Sorption processes and XRD analysis of a natural zeolite exchanged with Pb(2+), Cd(2+) and Zn(2+) cations. *J Hazard Mater* 2008; 156(1-3): 428-34.
- Wingenfelder U, Nowack B, Furrer G, Schulin R. Adsorption of Pb and Cd by amine-modified zeolite. *Water Res* 2005; 39(14): 3287-97.
- Dang VB, Doan HD, Dang-Vu T, Lohi A. Equilibrium and kinetics of biosorption of cadmium(II) and copper(II) ions by wheat straw. *Bioresour Technol* 2009; 100(1): 211-9.
- Sari A TMUOSM. Biosorption of Pb(II) and Ni(II) from aqueous solution by lichen (*Cladonia furcata*) biomass. *Biochemical Engineering Journal* 2007; 37(2): 151-8.
- Bhattacharyya KG, Gupta SS. Influence of acid activation on adsorption of Ni(II) and Cu(II) on kaolinite and montmorillonite: Kinetic and thermodynamic study. *Chemical Engineering Journal* 2008; 136(1): 1-13.
- Carvalho WA, Vignado C, Fontana J. Ni(II) removal from aqueous effluents by silylated clays. *J Hazard Mater* 2008; 153(3): 1240-7.
- Vijayaraghavan K, Lee MW, Yun YS. Evaluation of fermentation waste (*Corynebacterium glutamicum*) as a biosorbent for the treatment of nickel(II)-bearing solutions. *Biochemical Engineering Journal* 2008; 41(3): 228-33.
- Quintelas C, Rocha Z+, Silva B, Fonseca B, Figueiredo H, Tavares T. Removal of Cd(II), Cr(VI), Fe(III) and Ni(II) from aqueous solutions by an *E. coli* biofilm supported on kaolin. *Chemical Engineering Journal* 2009; 149(1-3): 319-24.
- Wan Ngah WS, Hanafiah MAKM. Adsorption of copper on rubber (*Hevea brasiliensis*) leaf powder: Kinetic, equilibrium and thermodynamic studies. *Biochemical Engineering Journal* 2008; 39(3): 521-30.
- Horsfall M, Ogban F, Akporhonor EE. Sorption of chromium (VI) from aqueous solution by cassava (*Manihot sculenta* Cranz.) waste biomass. *Chem Biodivers* 2006; 3(2): 161-74.
- Akhtar K, Akhtar MW, Khalid AM. Removal and recovery of uranium from aqueous solutions by *Trichoderma harzianum*. *Water Res* 2007; 41(6): 1366-78.
- Pradhan S, Singh S, Rai LC. Characterization of various functional groups present in the capsule of *Microcystis* and study of their role in biosorption of Fe, Ni and Cr. *Bioresour Technol* 2007; 98(3): 595-601.
- Barros MA, Machado N, Sousa-Aguiar E, Alves F, Pedroza S. Removal of Cr³⁺ from industrial and synthetic wastewater by naturally occurring clinoptilolite. In: Blanco J, Avila P, Editors. *Catalizadores y adsorbentes para la protección ambiental en la Región Iberoamericana*. Madrid, Spain: Cyted; 2001. p. 219-24. [In Spanish].
- Volesky B. Biosorption and me. *Water Res* 2007; 41(18): 4017-29.
- Pavan FA, Mazzocato AC, Jacques RA, Dias SLP. Ponkan peel: A potential biosorbent for removal of Pb(II) ions from aqueous solution. *Biochemical Engineering Journal* 2008; 40(2): 357-62.