Relationship Between Immobilization of Cells and Characterization of Binding Sites and of Cell-Free Bacteria and Immobilized Metal Biosorbents

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Research Article

Keywords: immobilization, binding sites, optimization, cell-free bacterial conditions for metal biosorbents, E. coli

Posted Date: December 17th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-1145236/v1

Abstract

Cell immobilization is preferred. Immobilized cells have been traditionally used for the treatment of sewage. The techniques employed for immobilization of cells are almost the same as those used for immobilization of enzymes with appropriate modifications. Entrapment and surface attachment techniques are commonly used. Gels, and to some extent membranes, are employed.

Certain microorganisms were found to amass metallic components at a high limit Was Known as Bacterial Biosorption, Potent metal biosorbents among microorganisms, at low pH esteems, cell divider ligands are protonated and contend essentially with metals for official. With expanding pH, more ligands, such as amino and carboxyl groups, could be exposed, leading to attraction between these negative charges and the metals and consequently incremental biosorption onto the cell surface. Starting with isolation and identification of heavy metal-resistant bacteria from rock ore. Studying Factors Affecting Uranium Biosorption, Optimization of bacterial growth conditions and optimum for metal uptake by free and immobilized bacterial cells. All this evidence suggest that functions groups Represented in our study are responsible for metal uptake in our bacterial biomass beside change in peaks position which assigned for its groups confirm biosorption of metal ions from waste due to ions charge interaction comparing with immobilized we found increase in no of binding sites indicate that immobilized bacterial have high efficiency for metal up take which also change in peaks position which assigned for its groups confirm biosorption of metal ions from waste due to ions charge interaction, Where the high biosorption yield obtained by bacteria.

Introduction:

Uranium sequestering (Biosorption).

Research is in progress to establish biosorption as a financially reasonable strategy to trap and accumulate metals. Biosorption can serve as a tool for the recovery of precious metals (e.g., from processing solutions or seawater) and for the elimination of poisonous metals (particularly from industrial wastewaters).

(SchiewerandVolesky2000). Adsorption and microprecipitation involve the binding of electrically neutral metals without the arrival of a stoichiometric amount of previously bound ions. In precipitation reactions, the main impetus is the interaction between the solute and the solvent, whereas the adsorption affinity among the sorbent and sorbate is the driving force. On account of the physicochemical mechanism by which interactions are based on physical adsorption, ion exchange, and complexion between metal and functional groups of the cell surface, metal binding does not depend on cellular metabolism**.** Tunali *et al.* (2006) indicated that the biosorption of lead and copper by Bacillus sp. involve a particle-exchange mechanism. Since the main mechanism involved in biosorption is ion exchange, protons compete with metal captions for the binding sites, and for this reason, pH is the operational condition that most strongly influences the process.

Immobilization (capsulation) of Bacteria

In addition to the high biosorption yield obtained by bacteria, the heavy metal bioremediation process requires microorganisms to be attached to a solid surface. Surface fixation and cell entrapment are the two methods of immobilization. Distinctive lattices were tested for cell immobilization **(Beolchini et al., 2003; Xiangliang et al., 2005).** Bolster frameworks appropriate for biomass immobilization incorporate alginate, polyacrylamide, polyvinyl liquor, polysulfone, silica gel, cellulose, and glutaraldehyde **(Wang 2002; Vijayaraghavan and Yun 2008a, b).** The polymeric grid determines the mechanical quality and synthetic protection of the last biosorbent molecule to be used for progressive sorption–desorption cycles, so it is imperative to pick the right immobilization lattice. **Akar et al. (2009**) measured the biosorption of 100 mgL−1 nickel at pH 6.5 to be 33.83 and 7.50 mgg−1 for silica gel and *Proteusvulgaris*, respectively, whereas the immobilized biosorbent had a biosorption capacity of 45.48 mgg−1 under the same conditions. Maximum biosorption obtained using immobilized biomass provides promise for immobilized cells in a column reactor for the remediation of heavy metals. At pH 5.0, the Cd^{2+} biosorption capacity of *E. coli* biomass-free PVA beads was 1.30 mgg−1, which was significantly lower than the adsorption capacity of PVA-immobilized cells, displaying capacities of 2.18 and 4.41 mg/g for biomass loadings of 8.42 and 19.5 wt %, respectively (Kao et al. 2009). Although cell entrapment imparts mechanical strength and resistance to chemical and microbial degradation upon the biosorbent, the costs of immobilizing agents cannot be ignored. Free cells are not suitable for use in a column due to their low density and size, and they tend to plug the bed, resulting in marked declines in pressure. For industrial applications of biosorption, it is important to utilize an appropriate immobilization technique to prepare commercial biosorbents that retain the ability of microbial biomass to adsorb metal(s) during the continuous treatment process. The immobilization of biomass in solid structures would create a biosorbent material with the right size, mechanical strength, rigidity, and porosity necessary for use in practical processes. Immobilized materials can be used in a manner similar to ion-exchange resins and activated carbon, such as adsorption–desorption cycles (i.e., recovery of the adsorbed metal, reactivation and reuse of the biomass) **(Veglio and Beolchini 1997).** In different matrices, the tested surface fixation was chosen as the immobilization methodology instead of cell entrapment. Cell immobilization has successfully been achieved mostly in calcium alginate beads, but this matrix also has a high affinity for heavy metals. Metal retention kinetics studies with calcium alginate confirmed that almost 100% of the metal assayed was retained by the beads (Vullo et al. 2003) and that it is pointless to try to improve heavy metal retention by bacterial cell entrapment in calcium alginate beads (Arica et al. 2001; Davis et al. 2003; Vullo et al. 2003; Arica et al. 2004). Although calcium alginate is useful for entrapping cells in its gel structure, its advantage resides mostly in the reutilization of the entrapped cells. However, the high heavy metal affinity of alginate makes it unusable for the development of continuous industrial processes, as the recovery of alginic acid would increase the final costs of effluent treatment.

Materials And Methods

1- Sampling

U-resistant bacterial strains were separated from the rock ore using nutrient agar (NA) medium and were prepared using peptic digestion of animal tissue (5 g/L), beef extract (3 g/L), NaCl (5 g/L.) and agar **15 g/L.**

2- Isolation and identification of heavy metal-resistant bacteria from rock ore.

The isolated metal-resistant bacteria were amended with different conc. Of U metal.

A pour plate was placed in NA medium and brooded at 37°C for 24 h.

3-Determination of heavy metal-resistant bacterial isolates by the plate diffusion method

Heavy metal-resistant bacteria were determined by the plate diffusion method (Hassen, *et al.* 1998). U solutions were prepared in different concentrations, i.e., 10, 20, 50, 100, 250, 500 and 1000 ppm. Each plate was spread with overnight societies of proper living beings. To each of the plates, 100 µl of appropriate U metal salt solutions were added to each well of 10 mm in diameter and 4 mm in depth. NA plates were incubated at 37°C for 24 h. After incubation, the zone of inhibition was measured.

A zone size less than 1 mm was scored as a resistance strain.

4. Optimization of bacterial growth conditions

Studying factor affecting bacterial growth Like pH., Temperature, the cultures were incubated at 37°C for 24,48 h and By Detecting O.D. The development was checked using a spectrophotometer (at 600 nm) 120 Min.

Preparation of *E. coli* Beads

We reported alginate–chitosan as an *E. coli* capsule.

The *E. coli* capsule/alginate–chitosan microcapsule was composed of E. coli sodium alginate, and calcium chloride. Therefore, under sterile conditions, the bacteria were mixed with sodium alginate solution, and then the mixed solution was dropped into calcium chloride solution for immobilization using microcapsule preparation instrument. *E. coli* capsule-loaded calcium alginate gel beads were obtained after immobilization, and the loaded calcium alginate gel beads were mixed with chitosan solution to obtain an E. coli capsule/alginate capsule system had good mechanical

strength, flexibility and biocompatibility between the *E. coli* capsule and the microcapsule. In addition, the internal three-dimensional network structure of the microcapsule provided sufficient space for E. coli capsule growth and good encapsulating stability (Kazy, et al 2006, Michaud, et al 2013).

6- Heavy metal adsorption by immobilized bacterial cells

The immobilized bacterial cells were set up as dabs agreeing with the strategy ofLeung *et al.* (2000**)** and were maintained in a conical flask containing 50 ml of samples for incubation, after which the specimens were pulled back for substantial metal examination by utilizing the titration method.

7. Cultivation of *E. coli.*

Cultivation of *E. coli.* The cells were placed in 250 mL cone-shaped flasks with 100 mL culture medium on a rotary shaker at 200 rpm at a constant temperature of 37°C. The culture medium contained the pH of the medium and was adjusted to (6).

8. Determination of Uranium:

The uranium content of the sample and prepared standard and treated solution were determined according to the method described by (**Davies &gray, 1964**).

9. Application of the FTIR spectra of U-loaded and unloaded free and immobilized cells & Scanning electron microscopy (SEM)

Preparation of immobilized *bacterial biomass* beads/alginate–chitosan microcapsules was composed of *E. coli* sodium alginate, chitosan and calcium chloride. Therefore, under sterile conditions, the bacteria were mixed with sodium alginate solution, and then the mixed solution was dropped into calcium chloride solution for immobilization using a microcapsule preparation instrument. *E. coli* capsule-loaded calcium alginate gel beads were obtained after immobilization, and the loaded calcium alginate gel beads were mixed with chitosan solution to obtain *E. coli* capsule/alginate–chitosan microcapsules. The microcapsule system had good mechanical strength, flexibility and biocompatibility between the *E. coli* capsule and the microcapsule. In addition, the internal three-dimensional network structure of the microcapsule provided sufficient space for *E. coli* capsule growth and good encapsulating stability.

Results & Discussion

1. Characterization Uranium-resistant bacterial isolates 1.1. Screening of uranium-resistant bacterial isolate growth in the presence of different Uranium conc. Isolated from Aborshid Uranyle rock sample.

Ten bacterial isolate 6 stable isolates were studied to select the most potent bacterial isolate to be utilized as a bacterial capsule in our study.

Table -1. Test for screening uranium-resistant isolates

Uranium conc. Isolate no.	100ppm	200ppm	300ppm	600ppm	1000ppm
S6	++	+	+	+	+
S4	+	+	+	+	+
S5	+	+	+	+	+
S7	+	+	+	+-	+-
S8	+-	-	-	-	-

Table 1 shows that we had 6-10 isolates tested for incubation with different conc. Of Uranium and investigate strong of growth against U conc. We found that the most potent isolate S6, S5. The growth continued with stability up to 1000 ppm uranium conc. This will continue with us

2.3. Techniques Used in Metal Biosorption Studies

In study of. **Jian-hua et al. (2007**) In investigation of. Jian-hua et al. (2007) effectively associated the number of acidic gatherings introduced to Bacillus cereus biomass, decided through potentiometric titrations, with the metal take-up limit. The idea of the coupling locations and their inclusion amid biosorption can be roughly assessed utilizing FTIR. Therefore, we utilized FTIR systems to determine the idea of the coupling locations where the investigation of **Mishra and Doble (2008)** indicated that carboxyl and amino groups were responsible for the binding of chromate. Carboxyl groups are negatively charged and abundantly available and actively participate in the binding of metal cations. **Kang et al. (2007)** observed that amine groups protonated at pH 3 and attracted negatively charged chromate ions via electrostatic interactions. Potentiometric titrations can provide information on the type and number of binding sites. **Kang et al. (2007**) titrated *Pseudomonas aeruginosa* and determined the p*K*a values of available binding sites **Loukidou et al. (2004).**

2.4. Analyzed the FTIR spectra of U loaded and unloaded

We used FT-IR spectra to confirm the availability of binding sites, as shown in Table 5. And data of FT-IR OF un loaded E. coli. (S6) unloaded and Bacterial Isolate as shown in table 2,3 For uranium, we found amino acid (O-H) stretching protein v (N-H) stretching, phosphate C-O stretching band, P-H stretching, protein amide I band mainly (C=O) stretching, protein (CH_2) and (CH_3) bending of methyl lipid (CH_2) bending of methyl, carbohydrate (c-o) of polysaccharides, nucleic acid (other phosphate containing

compound), >p=o stretching of phosphodiester, acid chlorides, C-Cl stretch in S6 *E. coli*, and comparing with dead isolates, we found the same beside acid chlorides at position 550 cm^{-1} C-Cl stretch **Cayllahua et al. (2009)**, who used FTIR spectra to confirm the presence of amide, carboxyl, and phosphate groups in *Rhodocs*. Biomass

Table: 2 data of FT-IR OF un loaded *E. coli*. (S6) unloaded and Bacterial Isolate.

Wavenumber range	Typical band	Intensity of loaded band live bacteria	Main peak(cm
3029-3639	Amino acid(O-H) stretching protein v(N-H) stretching	77.9	1-3439.42
2344-2365	Phosphate C-O Stretching band, P-H stretching	93	2-2355.62
1583-1709	Protein amide I band mainly(C=O) Streching	90	3-1638.23
1425-1477	Protien (CH_2) and (CH_3) bending of methyle Lipid (CH_2) bending of methyl	95	4-1428.99
1072-1356	Carbohydrate (c-o) of polysaccharides, Nucleic acid (other phosphate containing compound >p=o stretching of phosphodiester	100)	5-1101.15
730-550	acid chlorides C-Cl stretch	97	6-556.363

Table: 3 data of FT-IR OF unloaded *E. coli*. (S6) loaded Bacterial Isolate.

Main peak(cm^{-1})	Intensity of loaded band	Typical band	Wavenumber range
1-3455.42	50.4	Amino acid(O-H) stretching protein v(N-H) stretching	3029-3639
2-2088.62	99	Phosphate C-O Stretching band, PH stretching	2344-2365
3-1641.23	78	Protein amide I band mainly(C=O) Streching	1583-1709
4-1428.99	96	Protien (CH_2) and (CH_3)bending of methyle Lipid (CH_2) bending of methyl	1425-1477
5-1109.15	105	Carbohydrate (c-o) of polysaccharides, Nucleic acid (other phosphate containing compound) >p=o stretching of phosphodiester	1072-1356
6-593.363	92	acid chlorides CCl stretch	730-550
7-550.577	92	acid chlorides CCl stretch	730-550

A few band changes enabled the creators to anticipate the conceivable contribution of amino, carbonyl, carboxyl, and phosphate bunches in the biosorption of uranium.

Table: 4 data of FT-IR OF unloaded immobilized *E. coli.* (S6) Bacterial Isolate.

Main peak(cm^{-1})	Intensity of loaded band	Typical band	Wavenumber range
1-3436.42	71.14	Amino acid(O-H) stretching protein v(N-H) stretching	3029-3639
2-1725.23	97	Phosphate C-O Stretching band, P-H stretching	2344-2365
3-1636.3	92	Protein amide I band mainly(C=O) Streching	1583-1709
4-1380.99	86	Protien (CH_2) and (CH_3)bending of methyle Lipid (CH_2) bending of methyl	1425-1477
5-1354.15	80	Carbohydrate (c-o) of polysaccharides, Nucleic acid (other phosphate containing compound) >p=o stretching of phosphodiester	1072-1356
6-1038.48	91.90	Carbohydrate (c-o) of polysaccharides, Nucleic acid (other phosphate containing compound) >p=o stretching of phosphodiester	1072-1356
7-924.7.363	99	acid chlorides CCl stretch	730-550
8-879.577	99	acid chlorides CCl stretch	730-550
9-808.992	98	acid chlorides CCl stretch	730-550
10-597	88	acid chlorides CCl stretch	730-550

All this evidence suggests that functional groups presented in Tables 4, and 5, are responsible for metal uptake in our bacterial biomass. In addition, the change in peak positions assigned to its groups confirms the biosorption of metal ions from waste due to ion charge interactions. Comparing Tables 4, and 5 and 6 we found an increase in the number of binding sites, indicating that immobilized bacteria have high efficiency for metal uptake, which also changes the peak positions assigned to its groups, confirming the biosorption of metal ions from waste due to ion charge interactions.

Table 5 Data of FT-IR OF-loaded immobilized *E. coli*. (S6) Bacterial Isolates.

Main peak(cm^{-1})	Intensity of loaded band	Typical band	Wavenumber range
1-3909.42	96.64	Amino acid(O-H) stretching protein v(NH) stretching	3029-3639
2-3859.23	96	Phosphate C-O Stretching band, P-H stretching	2344-2365
3-1636.3	92	Protein amide I band mainly(C=O) Streching	1583-1709
4-1729.99	93	Protien (CH_2) and (CH_3)bending of methyle Lipid (CH_2) bending of methyl	1425-1477
5-1630.15	85	Carbohydrate (c-o) of polysaccharides, Nucleic acid (other phosphate containing compound) >p=o stretching of phosphodiester	1072-1356
6-1429.4	90.90	Carbohydrate (c-o) of polysaccharides, Nucleic acid (other phosphate containing compound) >p=o stretching of phosphodiester	1072-1356
7-1382.7	80	acid chlorides C-Cl stretch	730-550
8-1175.577	100	acid chlorides C-Cl stretch	730-550
9-1037.5.992	90	acid chlorides C-Cl stretch	730-550
10-936.27	103	acid chlorides C-Cl stretch	730-550
11-880.34	104.166	acid chlorides C-Cl stretch	730-550
12-818.634	101	acid chlorides C-Cl stretch	730-550
13-664.357	95	acid chlorides C-Cl stretch	730-550
14-562.148	92	acid chlorides C-Cl stretch	730-550

2.5. Energy dispersive X-ray (EDX)

Alginate beads (Fig. 1), predominantly ellipsoidal spheres, with an average diameter of 3–5 mm were used in the packed bed to remediate 10-1000 ppm U(VI) in a synthetic uranium solution. The effectiveness of different dosages of beads was considered, and the optimized ratio of 1:5 (v/v) of beads

to water was used in all batch studies of isotherm kinetics. Scanning electron microscopy of these beads, synthetic solution (Fig. 1), and control (Fig. 2), showed that they were hollow from inside (having smooth inner walls). In SEM/EDS analysis of the Ca-alginate beads after the experiment, void spaces of the beads were found to be filled with precipitates of heavy metals, showing that Ca-alginate beads can be successfully used as a biosorbent for the removal of uranium, which agreed with (Sumin Park and Minhee Lee 2017) in substantial metal evacuations, such as Cu and Cd. The picture of the control demonstrates the nonappearance of any metal besides uranium, which is not currently present. In Figs. 1, 2, 3, and 4, and table 6, uranium is indicated, as shown in Figs. 3 and 4. In the spot zone affirmed Uranium biosorption.

(Fig. 3). This established the fact that *E. coli* interacted with the metal present in the medium.

Kazy et al. (2006) utilized X-beam diffraction (XRD) examination and affirmed the inclusion of cell carboxyl and phosphate bunches in the authoritative form of lanthanum by Pseudomonas biomass. SEM micrographs supported analysts in dissecting cell surface morphology prior to and then afterward biosorption. **Tunali et al. (2006)** envisioned the surface of metal-stacked *Bacillus sp*. In our investigation, we utilized SEM to confirm U biosorption.

IR Examination of Cell wall of *E. coli*:

Cell wall of *E. coli* used in biosorption showing the functional group in the cell wall and comparing it with autoclaved forms exploring the difference in the intensity of the functional group of the cell wall involved in biosorption. This difference in intensity was reflected in biosorption capacity, as shown in Figure 4 functional group, and its intensity in the case of free cells.

2.7. Comparative study showing different forms of bacterial biomass.

Table: 6. Treatment for different forms of samples and applications.

	Immobilized Bacteria	Autoclaved bacteria	Free bacterial cell
Biosorption%			
Standard Uranium 100ppm	100%	80%	65%
Waste water (low conc.)	100%	100%	70%
Phosphoric acid(40PPM)	90%	50%	60%

4.3. Relation between Immobilization of Cells and efficiency of biosorption:

Immobilized individual enzymes can be successfully used for single-step reactions. They are, however, not suitable for multienzyme reactions and for reactions requiring cofactors. Whole cells or cellular organelles can be immobilized to serve as multienzyme systems. In addition, immobilized cells rather than enzymes are sometimes preferred even for single reactions due to the cost factor in isolating

enzymes. For enzymes that depend on the special arrangement of the membrane, cell immobilization is preferred. Immobilized cells have been traditionally used for the treatment of sewage. The techniques employed for immobilization of cells are almost the same as those used for immobilization of enzymes with appropriate modifications. Entrapment and surface attachment techniques are commonly used. Gels, and to some extent membranes, are employed.

Immobilized live Cells:

The viability of the cells can be preserved by mild immobilization. Such immobilized cells are particularly useful for fermentations. Sometimes mammalian cell cultures are made to function as immobilized viable cells.

In many instances, immobilized nonviable cells are preferred over enzymes or even viable cells. This is mainly because of the costly isolation and purification processes. The best example is the immobilization of cells containing glucose isomerase for the industrial production of high fructose syrup.

Conclusion:

Data showed that bacterial isolate can treat different forms of wastes and show that immobilized bacterial isolate gain high protection enable it to treat waste with high acidity like Phosphoric acid while free cell of the same bacterial isolate can not treat this type of waste so cannot complete biosorption process with the same efficiency so we found that immobilized bacterial isolates with biosorption efficiency reach 100% and 90%, respectively in case of standard Uranium, and Phosphoric acid, where immobilization Provide high number of active sites as mentioned above in Table 4, 5 entrapment imparts mechanical strength and resistance to chemical and microbial degradation upon the biosorbent, the costs of immobilizing agent cannot be ignored. Free cells are not suitable for use in a column; due to their low density and size, they tend to plug the bed, resulting in marked declines in pressure. For industrial applications, as in our study we made alginate beads like (capsule) of biosorption, it is important to utilize an appropriate immobilization technique to prepare commercial biosorbents, which retain the ability of microbial biomass to adsorb metal(s) during the continuous treatment process. The immobilization of biomass in solid structures would create a biosorbent material with the right size, mechanical strength, rigidity, and porosity necessary for use in practical processes.

Declarations

- **ETHICAL APPROVAL AND CONSENT TO PARTICIPATE**

Manuscripts Doesn’t reporting studies involving human participants,

- **CONSENT FOR PUBLICATION**

Not Applicable

- **AVAILABILITY OF DATA AND MATERIAL**

“Please contact author for data requests.”

- **COMPETING INTERESTS**

“The authors declare that they have no competing interests.”

- **FUNDING**

The authors have No Funds.

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Figures

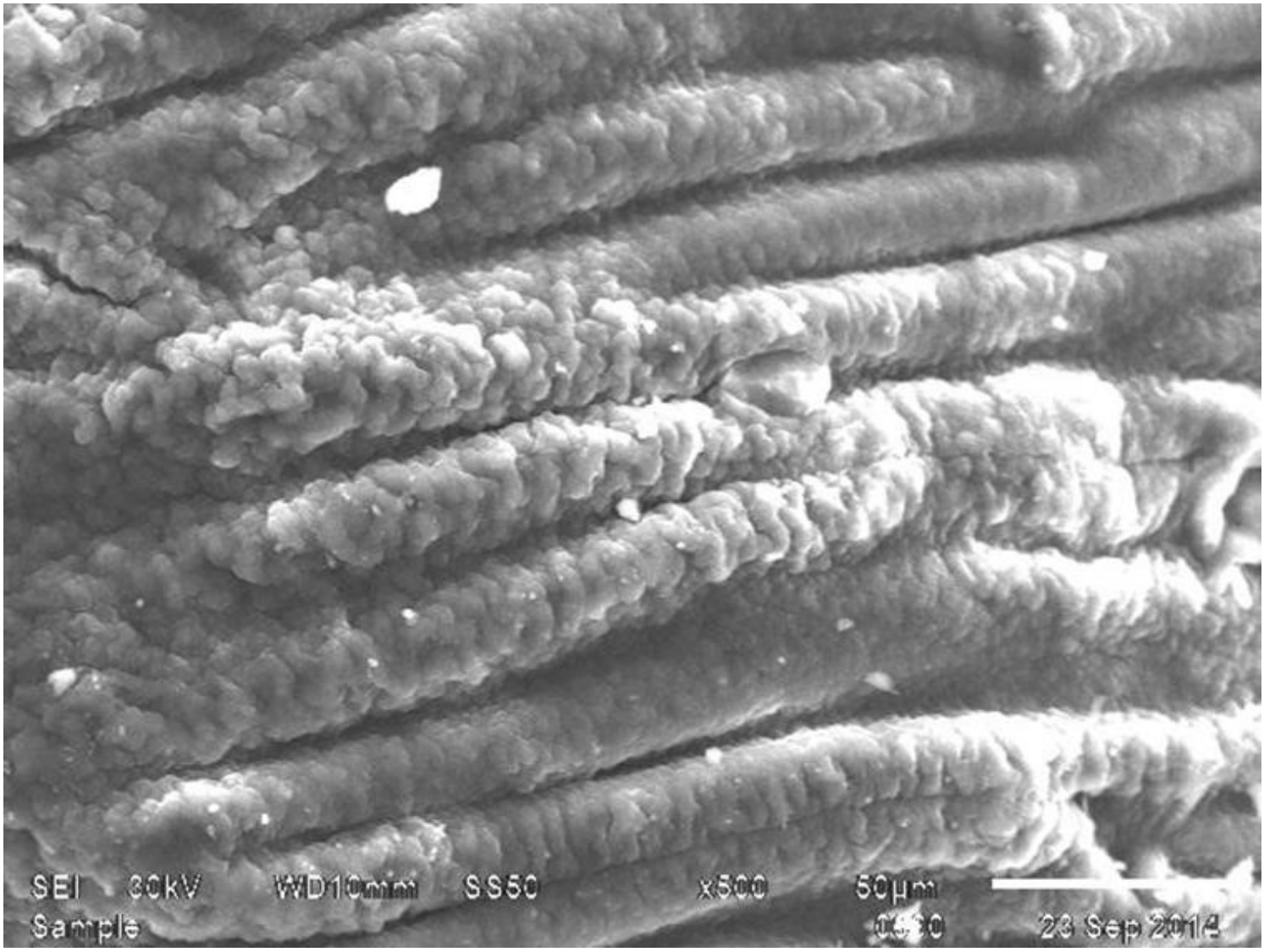


Figure 1

(EDX) to confirm the biosorption of U by capsulated cell-free extract-loaded Ca-alginate beads.

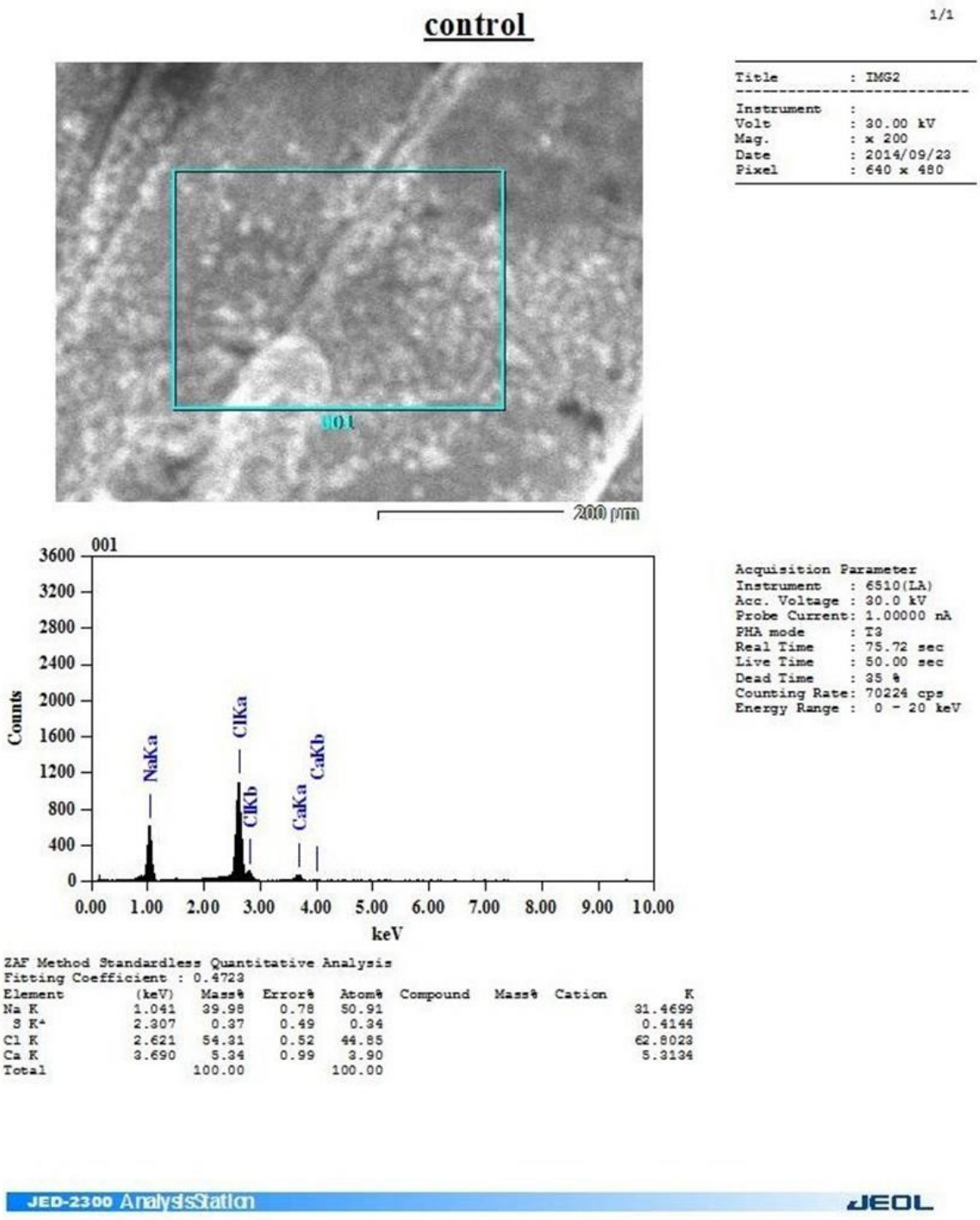


Figure 2

(EDX) to confirm Biosorption of U by capsulated Cell-Free Extract Loaded Ca-Alginate Beads

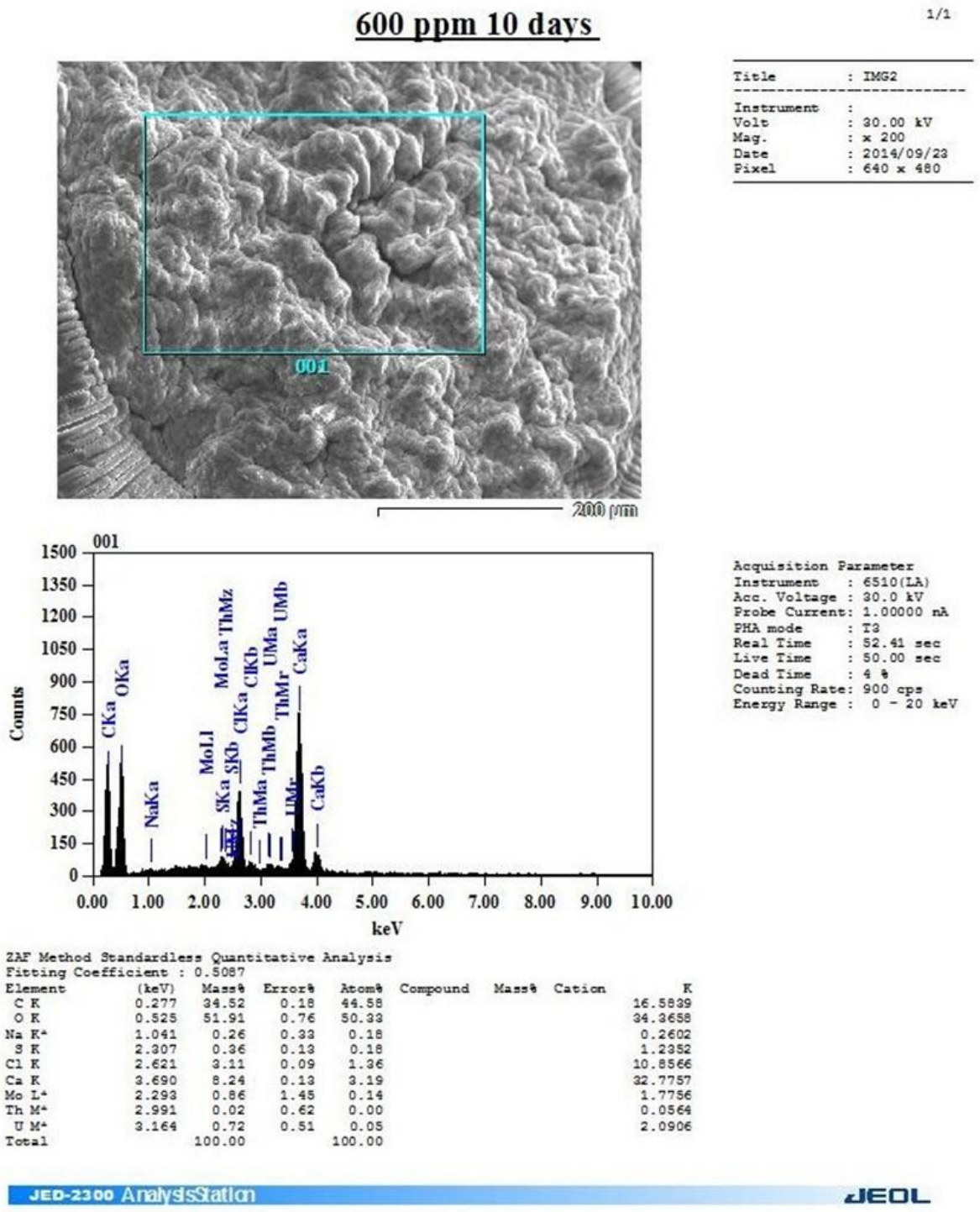
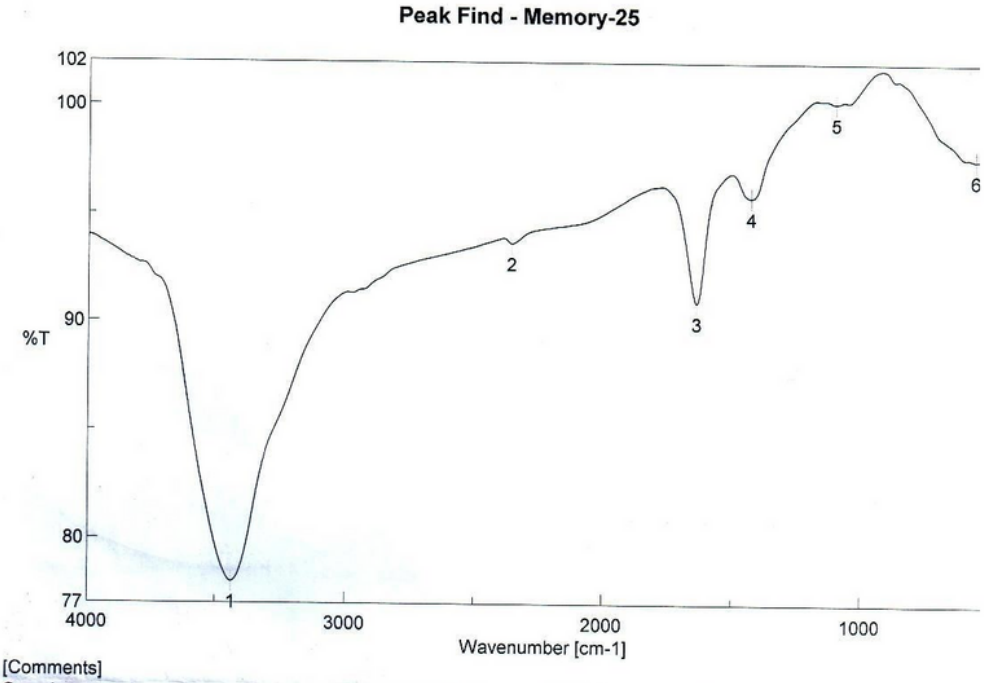


Figure 3

Bioremediation of 600 ppm U(VI) from synthetic solution in the bottle



[Comments]

Sample name	A
Comment	18/7/2017
User	IR
Division	IR
Company	MAC

[Result of Peak Picking]

No.	Position	Intensity	No.	Position	Intensity	No.	Position	Intensity
1	3439.42	77.9897	2	2355.62	93.6388	3	1638.23	90.9209
4	1428.99	95.7948	5	1101.15	100.155	6	556.363	97.5615

Figure 4

IR spectrum of live E. coli before the biosorption process.