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Comparative studies to remove cobalt ions from hazardous waste solutions by immobilized microbial species using several techniques: beads and thin film

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ABSTRACT

The study was conducted to use different microbial species isolated from low-level radioactive wastewaters (up to 2 mSv) for removing non-radioactive (Co(II)) and radioactive cobalt (Cobalt-60) from waste solutions using different immobilization techniques; beads and thin film. Simple and innovate technique was used to prepare calcium alginate thin film. The isolated bacterial species investigated were *Bacillus haynesii* and *Bacillus aerius* while fungal species were; *Aspergillus foetidus*, *Aspergillus parasiticus*, and *Penicillium oxalicum*. The bacterial species were immobilized in wet form, while the fungal species were immobilized in wet and dry forms using sodium alginate gel. Screening of bacterial and fungal species in free and immobilized shapes for Co(II) and Cobalt-60 removal percent and capacity was studied. Moreover, the efficient immobilized weight could be used in the different immobilization shapes was investigated. Batch experiments were used to determine the optimum environmental conditions for cobalt removal by the immobilized beads or thin film. Scanning electron microscopy was done to observe the surface changes. However, the energy-dispersive X-ray analysis results indicated the accumulation of cobalt ions on the surface. The immobilized dry *P. oxalicum* beads showed higher Cobalt-60 removal percent about 79.3% as compared to all other immobilized bacteria and fungi biomasses. The statistical analysis using Statistical Package of Social Science (SPSS) indicated that the studied bacterial species had significant high means with (p-value < 0.05) for the removal efficiency either free or immobilized than studied fungal species. Also, there was a significant high means for immobilization in thin film shape than immobilization in beads for removal of Co(II) in both bacteria and fungi. In contrast, the immobilized beads had a significant high mean for Cobalt-60 removal in both bacteria and fungi.

Keywords: Removal; Cobalt; Cobalt-60; Immobilization; Bacterial species; Fungal species; Beads; Thin film

1. Introduction

The contamination of the aquatic systems with toxic heavy metal ions recently has dramatically increased due to various human activities, such as agriculture, mining and other industrial processes [1]. The significant concentrations of these toxic elements in the environment ecosystems cause contamination of soil and water with deleterious impact [2–5]. Moreover, exceeding the threshold levels for these toxic elements have a detrimental effect on the microbial communities and their activities [6] and should be appropriately treated prior to disposal into rivers, seas, and land surfaces [7].

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Cobalt is considered a toxic heavy metal ion and its containing compounds are widely used in many industrial applications such as mining, electroplating, metallurgical, paints, pigments and electronic [8]. The cobalt concentration in the irrigation water and wastewater shouldn't exceed 0.05 and 0.1 mg L^{-1}, respectively [9]. Several health troubles are caused by the presence of cobalt in the environment such as; low blood pressure, vomiting, nausea, heart diseases, vision problems, sterility, thyroid damage, hair loss, bleeding, diarrhea and bone defects. It also causes mutations (genetic changes) in living cells [10]. The removal of Co(II) ions from the aqueous solution by a fast and effective process is necessary for environmental protection. Different techniques have been used for the removal of Co(II) ions such as; chemical precipitation, oxidation, ion exchange, reverse osmosis, membrane electrolysis, coagulation and adsorption [11–14]. Adsorption is a widely used technique because it is simple, adaptable, economical, and cost-effective.

Biosorption is a promising technology for pollutant streams treatment. It is based on metal–biomaterial interactions [15]. This process has advantages such as low cost of materials, ease of operation and selectivity against the alkaline metals when compared with an existing conventional physicochemical processes such as chemical precipitation, reverse osmosis, ion-exchange and activated carbon adsorption [16]. Fungal [17], algal [18,19], bacterial [20,21] and agricultural biomasses [22,23] have been employed for the biosorption removal of different pollutants from contaminated solutions.

Al-Fakih et al. [24] investigated the optimum environmental conditions for Pb(II) and Co(II) ions biosorption from aqueous solutions by *Saccharomyces cerevisiae* (*S. cerevisiae*) biomass treated with the NaOH. The maximum biosorption capacity of Co(II) ions was 11.69 ± 0.34 mg g^{-1}, at pH 7. Galedar and Younesi [25] studied the removal of cadmium, nickel and cobalt ions from aqueous solutions in batch and continuous systems using polysulfone immobilized ethanol pre-treated yeast, *S. cerevisiae*. Under optimum conditions, at pH 8, the maximum cobalt uptake capacity was 0.68 and 1.56 mg g^{-1} in batch and column studies, respectively. Polysulfone immobilization of yeast increased the removal by 48% compared with free ethanol pretreated yeast. Rani and Kaushik [26] reported the removal of hexavalent chromium Cr(VI) and Co(II) by immobilized dead cyanobacterial biomass of *Nostoc linckia* using different immobilization matrices. The immobilization matrices were; calcium alginate, polyvinyl alcohol-alginate and polyvinyl alcohol-alginate-glutaraldehyde. In a batch adsorption system, the maximum biosorption of Co(II) by immobilized beads of *Nostoc linckia* occurred at pH 3.0 in alginate biosorbent, while it was 5 in polyvinyl alcohol alginate and polyvinyl alcohol alginate glutaraldehyde biosorbent. Benmalek and Fardeau [27] reported that the bacterial strain 2YB-25OH isolated from wastewater showed the largest uptake percent 58.09%–79.41% of cobalt at pH 7.3–8.5.

Most of the biosorption research immobilized the biomaterial and microorganisms in the beads shape and very low attention has been bed to immobilization in sheets, screens and thin film. Previously, Kaya et al. [28] immobilized *Scenedesmus bicellularis* cells on alginate screens. Also, Zhang et al. [29] entrapped *Chlorella* sp. in calcium alginate as algal sheets to remove inorganic nutrients from domestic secondary effluents. In the present study, innovative, simple and low-cost technique was used to prepare the alginate thin film for immobilization of microbial species isolated from hazardous wastewaters. Screening of free and immobilized isolated species for their removal efficiency of Co(II) and Cobalt-60 from aqueous solutions was done in batch experiments. The effect of the immobilized shape and optimum conditions in the treatment process was investigated. Scanning electron microscopy (SEM) and energy-dispersive X-ray analysis (EDX) studies were done to investigate the surface of a plane (control) and immobilized beads or thin film and to indicate accumulation of the cobalt on the surface of the immobilized beads and thin film.

2. Materials and methods

2.1. Materials

All chemicals used were of analytical grades. A stock solution of $CoCl_2$ was prepared by dissolving distinct weight in one liter of bi-distilled water and other concentrations were prepared by dilution. The Cobalt-60 solution was prepared by spiking 10^{-3} M $CoCl_2$ solution with Cobalt-60 to obtain the desired activities. Sodium hydroxide and hydrochloric acid were used to adjust the pH of the solutions.

2.1.1. Isolates

Microorganisms were isolated from liquid radioactive wastewater collected from Waste Management Facility at Hot Laboratories Centre, Egyptian Atomic Energy Authority.

2.2. Methods

2.2.1. Isolation of bacterial and fungal species

In two sets each of three Petri-dishes, 1 mL of wastewater was mixed with nutrient agar and sabouraud agar to isolate bacteria and fungi, respectively, under sterile conditions. The nutrient agar plates were incubated at 37°C for 24 h, while the sabouraud agar plates were incubated at 30°C for 72 h. The obtained colonies were purified using a streak plate according to Benson [30]. The pure colonies were separated on nutrient agar or sabouraud slants for identifications.

2.2.2. Identifications of bacterial isolates

The bacterial isolates identification was carried out using 16S rRNA. The deoxyribonucleic acid (DNA) of the bacterial isolates was extracted using Amshag kit. The extracted DNA was checked through the migration in 1% agarose gel using 1X TBE (Tris-Borate-EDTA) running buffer followed by photographing using a gel documentation system (Syn. Gene, UK). The amplification of the bacterial isolates 16S rRNA gene was achieved using universal primers F984 (5′-AACGCGAAGAACCTTAC-3′), and R1378 (5′-CGGTGTGTACAAGGCCCGGGAACG-3′), according to [31], with some modifications. The polymerase chain reaction (PCR) program was as follows 1 min at 96°C, followed by 30 cycles at 94°C for 1 min, 55°C for 1.5 min, 72°C for

2 min, using maximal ramp rates throughout, with the final 72°C segment of the cycle extended to 6 min before cooling to 25°C. The PCR products were detected through migration in 1% agarose gel, in 1X TBE buffer. The gel was then examined, using a gel documentation system. The rest of the amplified PCR products were then submitted for a nucleotide sequencing process (Sigma, Germany). Moreover, the obtained sequences were deposited in GenBank with new accession numbers [32].

2.2.3. Identifications of fungal isolates

The fungal species were identified using the identification program of the image analysis system [33] at the Regional Center for Mycology and Biotechnology (RCMP), Al-Azhar University, Egypt.

2.2.4. Biomass production

The pure bacterial and fungal slants were eluted with sterilized 5 mL distilled water and transferred to 500 mL conical flasks containing 150 mL nutrient or sabouraud broth, respectively. Then flasks were incubated in an environmental orbital shaker at 37°C and 27°C for 24 h, respectively. Each conical flask was used to inoculate three one liter conical flasks containing 200 mL of nutrient or sabouraud broth, respectively. The produced biomasses were harvested using a cooling centrifuge, washed with distilled water, centrifuged again and then kept frozen as bulk biomass till use [34,35].

2.2.5. Immobilization techniques

2.2.5.1. Preparation of control and immobilized beads

2.2.5.1.1. Calcium alginate beads

Calcium alginate beads were prepared by dissolving 4 g sodium alginate in 100 mL distilled water with gentle stirring overnight. Then the gel was injected by the peristaltic pump into the 100 mL caustic solution of 2% $CaCl_2$. The formed beads were left in $CaCl_2$ solution for 1 h with gentle stirring, then washed thoroughly with distilled water and kept in the refrigerator for use Plate 1a according to Omar et al. [36].

2.2.5.1.2. Immobilized calcium alginate beads

The immobilized wet bacterial and dry fungal biomasses beads were prepared by mixing the optimum biomass weight thoroughly with a certain volume of 4% sodium alginate gel. On the other hand, the exact wet fungal biomass was taken and ground before mixing with 4% sodium alginate gel. The biomass-alginate mixture was kept in the refrigerator for 15 min and then injected into 2% $CaCl_2$ solution Plate 1b–d. The beads had a diameter of 2.5–3.0 mm. Every 10 mL alginate gel and biomass alginate gel gave about 500 beads. The obtained beads were kept in a refrigerator for use [35].

2.2.5.2. Preparation of control and immobilized thin film

A new innovative and simple technique was established and used for the preparation of thin-film either control or immobilized with biomass. On a glass plate, 5 mL of the gel was poured inside a fixed circle its edge determined the thin film thickness. Filter paper wetted with 2% $CaCl_2$ was put over the film for 30 min until solidification. Then the film transferred to petri-dish containing 2% $CaCl_2$ for 30 min. Then the produced film was washed with bi-distilled water. The control thin film was prepared for sodium alginate gel only Plate 1a, while the immobilized thin film prepared by mixing sodium alginate gel with biomass prepared as mention above Plate 1b–d. The control and immobilized thin film were then cut into cubes of 0.5 mm side length Plate 1a–d.

2.2.6. Determination of Co(II) metal ions

Atomic absorption spectrophotometer (Buck Scientific) was used to measure cobalt concentrations at wavelengths 240 nm. Standard solutions at different concentrations were used for standard curve measurements. The concentration was given directly by the instrument.

2.2.7. Determination of Cobalt-60

The activity of the Cobalt-60 solution was determined by recording the integral area under Cobalt-60 peaks 1,173.2 and 1,332.5 Kev, where 2 mL samples were counted for 600 s (10 min) using sodium iodide crystal connected to a multichannel analyzer. The instrument was first calibrated with point source and the main of triplicate measurements was taken.

2.2.8. Removal experiments

Removal experiments were done in the batch experiment and under optimum conditions; pH value of 6.0 ± 0.5, at temperature 25°C ± 3°C with stirring of 120 rpm. The exact weight of bacterial and fungal-free biomasses or immobilized in two shapes (beads and thin-film) was mixed with a certain volume of Co(II) and Cobalt-60 solution with respective concentration and activity, in 100 mL conical flask. The experiments were followed and the concentrations or activities were determined at different time intervals.

2.2.9. Calculations

The biosorption capacity (q_e) at equilibrium was calculated from the relationship.

$$q_e = \frac{\left(C_0 - C_e\right) V}{m} \tag{1}$$

where C_0 and C_e are the initial and equilibrium concentrations of Co(II) ions in aqueous solution (mg), respectively. V and m are the volumes of solution (L) and dry weight (g) of adsorbent (free biomass, control gel or immobilized system), respectively.

The Co(II) removal percent (R %) was calculated from the equation:

$$R\ \% = \left[\frac{\left(C_0 - C_e\right)}{C_0}\right] \times 100 \tag{2}$$

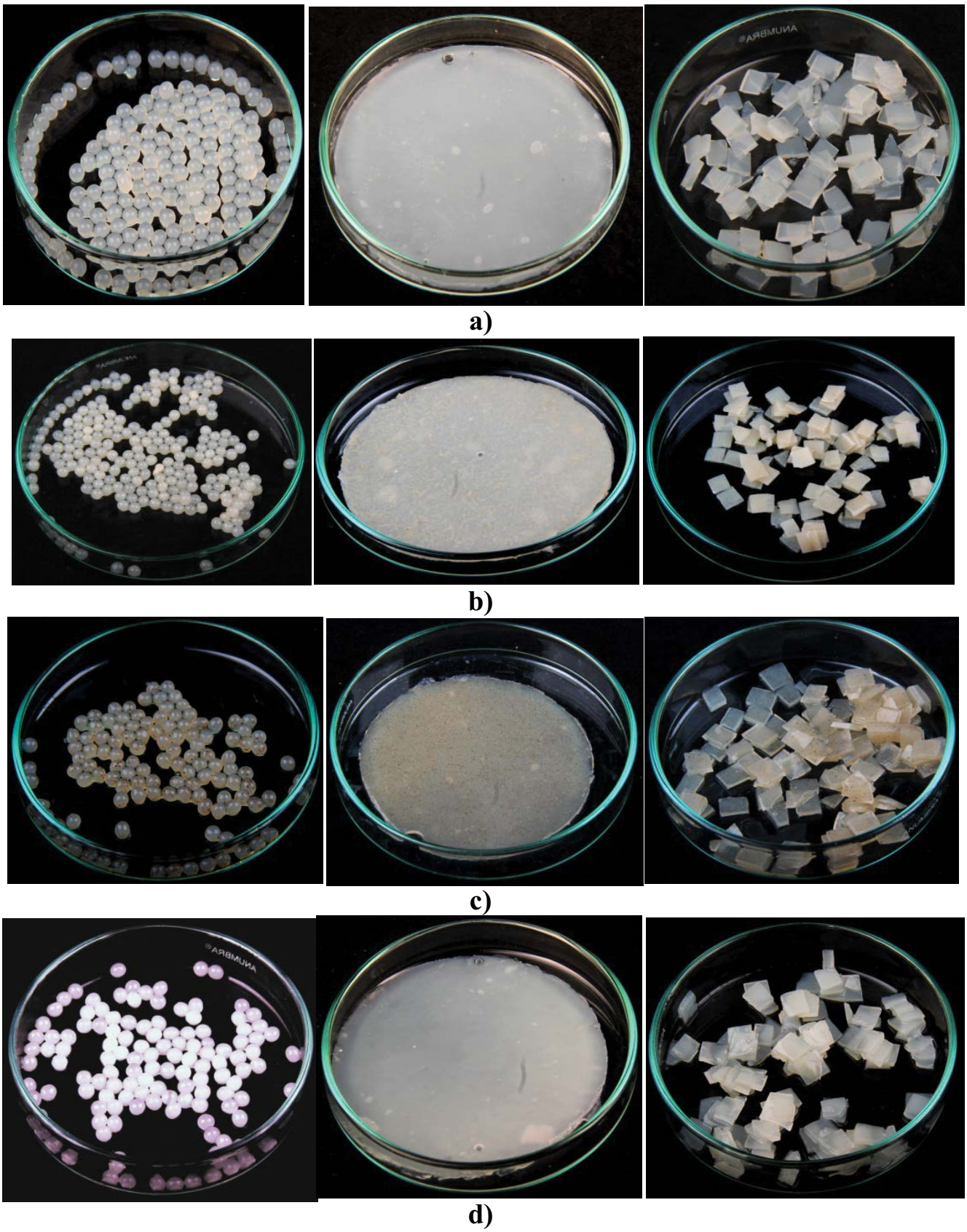


Plate 1. (a) Control calcium alginate beads and thin film, (b) immobilized wet fungal biomass beads and thin film, (c) immobilized dry fungal biomass beads and thin film, and (d) immobilized wet bacterial biomass beads and thin film.

The activity of Cobalt-60 solution was determined from the equation:

$$A = E \times I \tag{3}$$

where (A) is the activity, (E) is the efficiency of the system and (I) is the intensity of element.

The removal percent of Cobalt-60 at different contact times:

$$R\ \% = \left[\frac{\left(A_0 - A_e\right)}{A_0}\right] \times 100 \tag{4}$$

where A_0 and A_e are the activity at initial and equilibrium, respectively.

The activity Q (Bq ml^{-1}) removed by g dry weight:

$$Q = \frac{\left(A_0 - A_e\right)V}{m} \tag{5}$$

where (Q) is the activity biosorption capacity and Bq (Becquerel) is an activity unit equal to disintegrations per second [36].

2.2.10. Statistical analysis

Data analysis was performed using the Statistical Package of Social Science (SPSS) software version 18 in windows 7. For quantitative parametric data; an independent student *t*-test was used to compare measures of two independent groups of quantitative data [37]. One way ANOVA test was used to compare more than two independent groups of quantitative data [38].

For quantitative non-parametric data; the Kruskal-Wallis test was used in comparing more than two independent groups [39]. Mann-Whitney test in comparing two independent groups [40]. The p-value ≤ 0.05 was considered the cut-off value for significance.

3. Results and discussion

3.1. Identifications of bacterial isolates

The nucleotide sequences were submitted to the GenBank, to confirm their identity. The isolated bacterial species were identified as; *Bacillus aerius* (*B. aerius*) AEM and *Bacillus haynesii* (*B. haynesii*) AEA with accession numbers MT322818 and MT322819 [32].

3.2. Identifications of fungal isolates

According to the gross morphology include, the rate of growth, colony diameter, texture, color and reverse pigmentation as well as the measurements of the diagnostic structures that characterized the isolated species [33,41–43]. The fungal species were *Aspergillus foetidus* (*A. foetidus*), *Aspergillus parasiticus* (*A. parasiticus*) and *Penicillium oxalicum* (*P. oxalicum*).

3.3. Removal of cobalt ions

3.3.1. Screening of the free wet biomasses of bacteria and fungi

Different free biomasses weights of both bacteria and fungi were examined for the removal of cobalt ions. Results showed that the removal capacity was 400, 356 mg Co(II) g^{-1} dry weight of *B. haynesii* and *B. aerius*, respectively. On the other hand, fungal biomass of *A. foetidus*, *A. parasiticus* and *P. oxalicum* had removal capacities 96, 196.8 and 139.2 mg g^{-1}, respectively. Besides, the bacterial biomass had higher removal capacities even it had lower or the same removal percent of the fungal biomass Table 1. This was attributed to the bigger surface area to volume ratio of the bacterial cells compared with that of fungal cells. As the free biomass increased the removal percent increased and the capacity decreased [36]. The maximum capacity was obtained at 0.025 g free wet biomass for both bacteria and fungi. Colagar et al. [44] reported that maximum Co(II) ions removal capacity by a Cyanobacterium, *Oscillatoria* sp. isolated from Mazandaran River, Iran was 30.12 mg g^{-1} dry biomass. Also, Gharieb et al. [45] showed that at metal ion concentration 200 mg L^{-1}, the maximum saturation of NaOH pretreated *Rhizopus oryzae* was 69.73 and 13.56 mg g^{-1} for Pb(II) and Co(II), respectively.

3.3.2. Screening of the free dry biomasses of fungi

The dry biomasses of fungi were examined for their removal capacities at different weights. The removal capacities were 272, 298 and 152 Co(II) g^{-1} dry weight of *A. foetidus*, *A. parasiticus* and *P. oxalicum*, respectively. The maximum capacity was achieved at free dry biomass 0.005 g. The increase in the free dry biomass weight increased the removal percent and decreases capacity in Table 2. Results showed that the free dry mycelia had high efficiency than free wet mycelia. Hajahmadi [46] investigated competitive biosorption of multicomponent heavy metals Zn(II), Co(II) and Cd(II) from aqueous solution by pretreated dried

Table 1
Screening of the free wet biomass of bacteria and fungi with different weights for removal of Co(II) 250 ppm, after 2 h

Biomass weight (g)	Co(II) removal %					Capacity Co(II)/free biomass dry weight (mg g^{-1})				
	Bacteria		Fungi			Bacteria		Fungi		
	B. haynesii	*B. aerius*	*A. foetidus*	*A. parasiticus*	*P. oxalicum*	*B. haynesii*	*B. aerius*	*A. foetidus*	*A. parasiticus*	*P. oxalicum*
0.025	8	7.12	4.80	9.84	6.96	400	356	96	196.8	139.20
0.050	9	8.16	5.00	10.24	7.50	225	204	50	102.4	75.00
0.100	14	10.20	6.72	11.00	9.36	175	127	33.60	55.0	46.80
0.200	15.2	13.30	8.50	11.80	10.50	95	83	21.25	29.5	26.25

Table 2
Screening of the free dry biomass of fungi with different weights for removal of Co(II) 250 ppm, after 2 h

Biomass weight (g)	Co(II) removal %			Capacity Co(II)/free biomass dry weight (mg g^{-1})		
	A. foetidus	*A. parasiticus*	*P. oxalicum*	*A. foetidus*	*A. parasiticus*	*P. oxalicum*
0.005	10.88	11.92	6.08	272	298	152
0.010	8.00	9.20	8.50	100	115	106.25
0.020	10.40	12.00	8.24	65	75	51.50
0.025	11.84	13.40	5.04	59.2	25.2	25.20

Aspergillus niger in a batch system. It was found that as the initial concentration increased from 10 to 300 mg L^{-1}, adsorption capacity increased from 6.15 to 31.68 for Co(II) ions.

3.3.3. Screening of the immobilized wet biomasses of bacteria

Screening of the immobilized wet biomasses of the isolated bacteria; *B. haynesii* and *B. aerius* showed that the removal percent and capacity were nearly the same for both beads and thin-film shapes. Also, it was cleared that the optimum immobilized wet biomasses for both beads and thin film form was 0.025g/5 mL sodium alginate gel. The removal percent and capacity of wet *B. haynesii* immobilized thin film were 48.48% and 37.50 mg Co(II) g^{-1} dry weight, while those of wet *B. aerius* immobilized thin film were 46.32% and 35.38 mg Co(II) g^{-1} dry weight Table 3. Galedar and Younesi [25] used polysulfone immobilized ethanol pretreated yeast, *S. cerevisiae*, for removal of cadmium, nickel and cobalt ions from aqueous solutions. It was found that the uptake capacity of the metal ions was 3.74 mg g^{-1} for cadmium, 1.57 mg g^{-1} for nickel and 1.56 mg g^{-1} for cobalt.

3.3.4. Screening of the immobilized wet and dry biomasses of fungi

Results showed that the immobilized wet biomasses of *A. foetidus* and *P. oxalicum* thin film had high removal capacity 35.20 and 35.38 mg Co(II) g^{-1} dry weight, respectively, than the beads forms 29.70 and 34.22 mg Co(II) g^{-1} dry weight, respectively. In contrast, the immobilized wet biomass of *A. parasiticus* both beads and thin-film forms had nearly the same removal percent and capacity. On the other hand, for the immobilized dry biomass the beads form showed higher removal percent and capacity for all studied fungal species. The higher removal percent and capacity 58.40% and 44.24 mg Co(II) g^{-1} dry weight were achieved by dry immobilized beads of *A. parasiticus*. Also, results showed that the optimum immobilized wet biomass was 0.025 g/5 mL sodium alginate gel for beads and thin film. However, the optimum immobilized dry biomass was 0.01 g/5 mL sodium alginate gel for both beads and thin-film Table 4. El-Morsy et al. [47] revealed a marked increase in uptake of all tested metals by the alkali-treated alginate-immobilized *Mucor racemosus* biomass over free biomass. The maximum uptake was 111.58 mg Zn g^{-1} at 100 ppm, 121.33 mg Cu g^{-1} at 150 ppm and 96.15 mg Pb g^{-1} at 100 ppm.

3.3.5. Effect of environmental conditions

The optimum environmental conditions for the removal of Co(II) ions were studied using the optimum immobilized shapes (beads and/or thin-film) of the studied microorganisms. For bacteria, immobilized 0.025 g/5 mL gel *B. haynesii* beads and thin-film were examined. Whereas, thin-film of immobilized 0.025 g/5 mL dry *A. foetidus* and beads of immobilized 0.01 g/5 mL dry *A. parasiticus* were studied for fungi.

The immobilized wet biomass of *B. haynesii* in either beads or thin-film shapes showed relatively the same affects at different pH values. For immobilized dry *A. foetidus* thin-film and immobilized dry *A. parasiticus* beads, the effect of pH values has the same pattern with high removal for beads shape (Fig. 1). The dry immobilized fungi showed higher removal percent than immobilized bacteria at low and high pH. The rate of increase in removal percent with the increase in pH for immobilized bacteria is greater than that of fungi (Fig. 1). The maximum removal percent achieved at pH 6.5 for both studied bacteria and fungi. Al-Fakih et al. [24] reported that pH plays a major role, where maximum

Table 3
Screening of the bacterial species immobilized at different weights and shapes for the removal of Co(II) 250 ppm, after 2 h

Immobilized weight (g)	Co(II) removal %				Capacity Co(II)/Immobilized biomass dry weight (mg g^{-1})			
	B. haynesii		*B. aerius*		*B. haynesii*		*B. aerius*	
	Beads	Thin film	Beads	Thin film	Beads	Thin film	Beads	Thin film
0.010	48.16	46.56	41.92	46.80	37.48	36.24	32.63	36.43
0.025	47.28	48.48	44.00	46.32	36.57	37.50	34.03	35.83
0.050	42.88	40.80	42.16	48.08	32.80	31.21	36.78	40.32
0.100	41.52	41.76	43.36	46.08	31.15	32.33	32.53	34.57

Table 4
Screenings of the fungal species (wet and dry) immobilized at different weights and shapes for removal of Co(II) 250 ppm, after 2 h

Adsorbent used	Weight (g)	Co(II) Removal %				Capacity Co(II)/Immobilized biomass dry weight (mg g^{-1})			
		Beads		Thin Film		Beads		Thin Film	
		Wet	Dry	Wet	Dry	Wet	Dry	Wet	Dry
	0.005	32.40	36.08	39.20	44.30	25.25	27.75	30.60	33.90
	0.010	38.16	54.32	44.96	50.08	29.70	41.15	34.91	36.82
A. foetidus	0.025	38.48	53.28	45.76	50.72	29.70	38.61	35.20	34.27
	0.050	35.44	51.60	50.24	51.60	27.11	34.86	30.06	30.71
	0.100	37.44	50.88	41.36	51.44	28.09	30.29	30.41	24.73
	0.005	34.00	33.68	35.40	44.70	26.50	25.91	27.60	34.20
	0.010	41.36	58.40	40.80	48.56	32.19	44.24	31.68	35.71
A. parasiticus	0.025	44.80	56.20	44.72	47.20	34.65	40.72	34.40	31.89
	0.050	39.20	54.40	40.16	50.88	29.99	36.76	30.42	30.29
	0.100	38.40	54.16	45.12	47.52	28.81	32.24	33.18	22.85
	0.005	34.64	32.48	36.50	44.70	27.00	24.98	28.50	32.80
	0.010	38.84	55.84	38.80	45.04	31.01	42.30	30.19	34.46
P. oxalicum	0.025	44.24	55.36	46.00	49.52	34.22	40.12	35.38	33.46
	0.050	42.16	54.16	46.72	51.28	32.25	36.60	35.39	30.52
	0.100	42.64	52.24	45.36	48.24	31.99	31.10	33.35	23.19

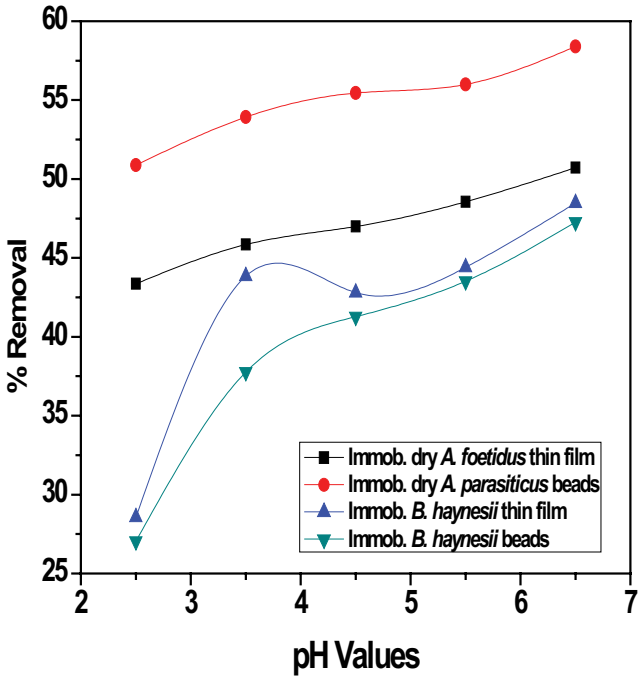


Fig. 1. Effect of pH values on the removal percent of Co(II) ions 250 ppm using immobilized adsorbents beads and thin films.

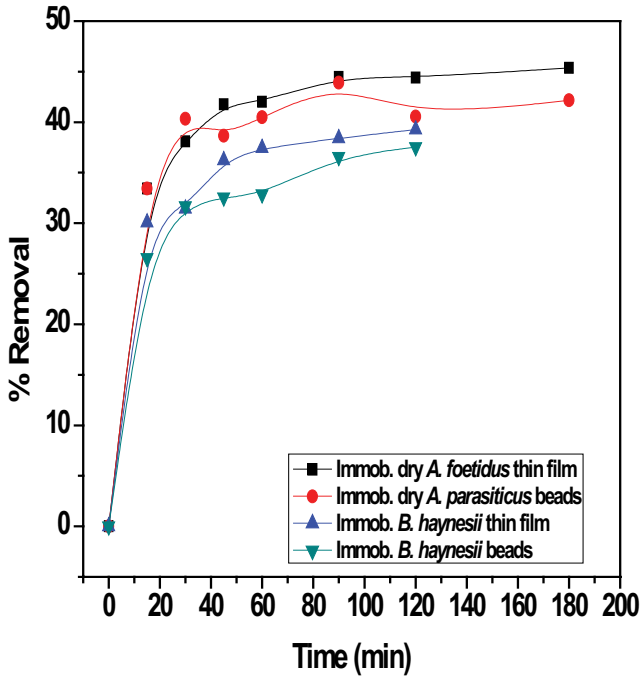


Fig. 2. Effect of time on the removal percent of Co(II) ions 250 ppm using immobilized adsorbents beads and thin films.

Co(II) biosorption capacities using fungal biomasses were 6.20 ± 0.32 mg g^{-1} at pH 7. The equilibrium for both bacteria and fungi in the studied immobilized shapes was found after 90 min (Fig. 2). Rani and Kaushik [26] stated that the biosorption equilibrium of cobalt removal using alginate immobilized biomass of *Nostoc linckia* occurred at 90 min of contact time, followed by leakage of adsorbed metal ions and then became constant [48].

The effect of temperature on immobilized *B. haynesii* either beads or thin-film showed the same pattern, where the highest removal percent and capacity were showed at 25°C. The highest removal percent was up to 25°C for immobilized

beads of dry *A. parasiticus* and up to 37°C for immobilized thin film of dry *A. foetidus* (Fig. 3). Park et al. [49] indicated that the increase in temperature increased biosorption of heavy metal by increasing biosorbent surface activity and kinetic energy of the adsorbate, but temperature over 50°C causes physical damage of biosorbent structure. Hemambika et al. [50] observed that removal of Co(II) using immobilized fungi increased with an increase in the temperature range from 20°C to 40°C and further increases decreased ions adsorption.

In general, an increase in Co(II) ions concentration resulted in a decrease in removal percent and an increase in the removal capacity [36]. The highest removal capacity was achieved at 750 ppm for an immobilized thin film of *B. haynesii* and thin-film of dry *A. foetidus* (Fig. 4). At low concentrations the active sites on the adsorbent are abundant, therefore, uptake capacity will increase rapidly initially. Then at high concentrations, adsorption sites were saturated resulted in approximately constant uptake capacity [26,51].

3.4. Removal of Cobalt-60

3.4.1. Screening of the immobilized wet biomasses of bacteria and fungi

Results showed that immobilized beads achieved higher removal percent and capacity than thin film for Ca-alginate control and immobilized with bacteria or fungi. Also, results indicated that the immobilized beads (0.1 g/5 mL gel) of *B. haynesii* had higher removal percent 53.4% than *B. aerius* 51.2%. For fungi, the immobilized beads (0.02 g/5 mL gel) of dry weight *P. oxalicum* showed a higher removal percent 79.3% than the wet or dry mycelia of other fungi (*A. foetidus* and *A. parasiticus*) immobilized in different immobilized shapes Table 5. Sasaki et al. [52] used alginate immobilized beads of *Rhodobacter sphaeroides* SSI in practical removal of radioactivity from polluted soil in Fukushima, Japan. The mesh bags of beads were able to decrease the radioactivity from the suspension of 5 kg of soil/10 L of tap water by 31% after 15 d under aerobic treatment. When treatment combined with anaerobic fermentation for 5 d the radioactivity decreased by 66%.

3.4.2. Effect of the immobilized weight

It was necessary to determine the optimum immobilized weight that could be used to achieve higher radioactivity removal percent and capacity. The optimum immobilized wet weight of *B. haynesii* was found to be 0.2 g/5 mL sodium alginate gel, while the optimum immobilized dry weight of *P. oxalicum* was 0.02 g/5 mL sodium alginate gel (Figs. 5 and 6).

3.4.3. Effect of different Cobalt-60 activities

The results obtained using the activity of 15,000 count/600 s showed the highest removal percent 79.3% and 53.4% for immobilized beads of dry *P. oxalicum* and immobilized beads of wet *B. haynesii*, respectively. As the activity increased from 20,000 to 25,000 count/600 s the removal percent of immobilized beads of dry *P. oxalicum* was nearly the same as the immobilized beads of wet *B. haynesii* (54%/56%–58%/57.4%), respectively. Wherever the removal percent of the control alginate beads was slightly increased from 40% to 43.9% with the increase of activity from 15,000 to 25,000 count/600 s (Fig. 7).

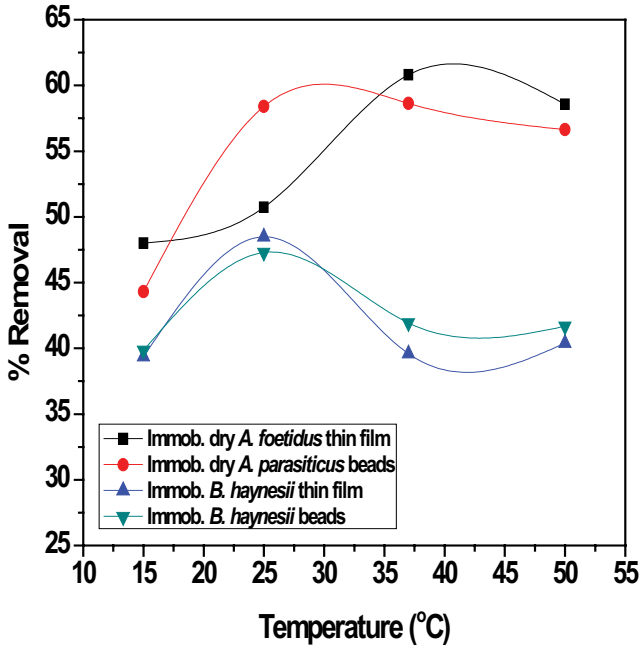


Fig. 3. Effect of temperatures on the removal percent of Co(II) ions 250 ppm using immobilized adsorbents beads and thin films.

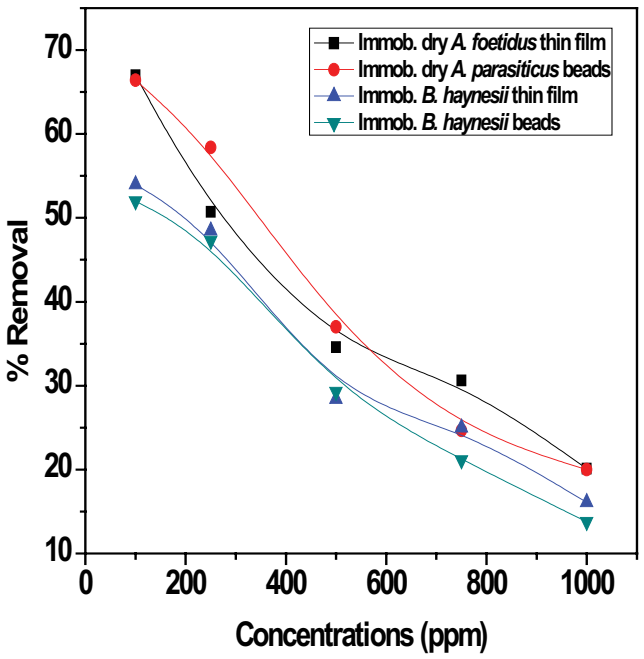


Fig. 4. Effect of Co(II) ion concentrations on the removal percent of Co(II) using immobilized adsorbents beads and thin films.

Table 5
Screening of calcium alginate; control and immobilized with bacterial or fungal species at different weights and in different shapes for removal of Cobalt-60 (15,000 count/600 s)

			Cobalt-60 removal %		Capacity Cobalt-60/adsorbent dry weight (mg g^{-1})	
			Beads	Thin film	Beads	Thin film
Control			40.0	33.8	5.10	4.30
Bacteria	*B. haynesii*	0.10 w	53.4	41.4	6.48	5.86
	B. aerius	0.10 w	51.2	39.8	6.21	5.63
Fungi	*A. foetidus*	0.10 w	47.2	36.8	5.73	5.21
		0.02 d	62.0	45.6	7.53	5.30
	A. parasiticus	0.10 w	51.0	37.4	6.18	5.29
		0.02 d	67.9	51.0	8.20	5.90
	P. oxalicum	0.10 w	59.5	40.0	7.22	5.66
		0.02 d	79.3	52.1	9.62	6.00

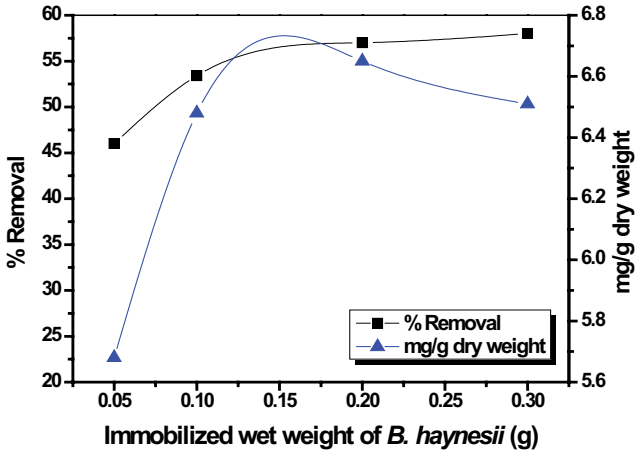


Fig. 5. Removal percent and capacity of Cobalt-60 for immobilized wet weight beads of *B. haynesii*.

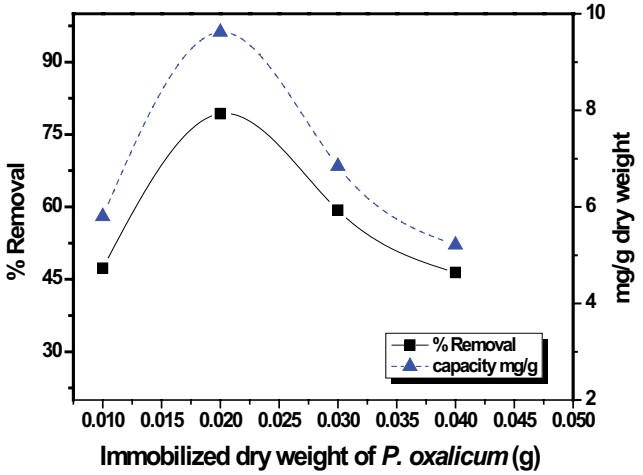


Fig. 6. Removal percent and capacity of Cobalt-60 for immobilized dry weight beads of *P. oxalicum*.

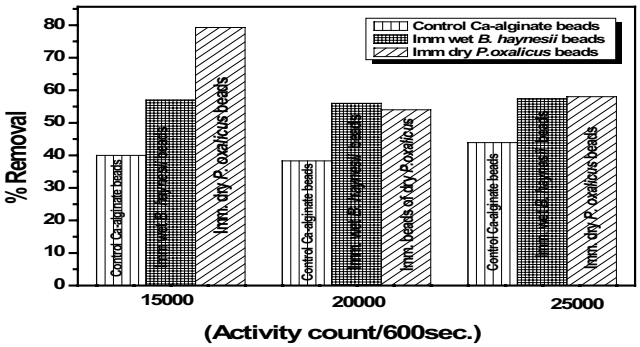
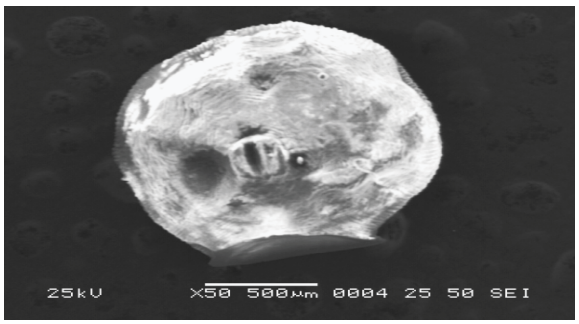


Fig. 7. Effect of different Cobalt-60 activities on the removal percent by the different adsorbents.

3.5. SEM analysis

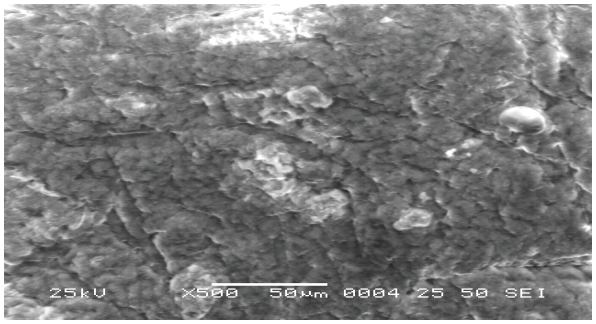
The scanning electron microscope images were done using JEOL scanning electron microscope (JSM-5600LV, Japan) for beads and thin films after drying at room temperature. Plate 2a showed the plain calcium alginate (CA) beads at low magnification (X35). Plain CA-beads before and after removal of cobalt with high magnification power (X500) were shown in Plate 2b and c. Plate 2d and f showed the CA-beads immobilized with bacteria and fungi and to illustrate the change in the surface by immobilization of microorganisms. On the other hand, the changes in the immobilized beads due to the accumulation of cobalt ions were illustrated in Plate 2e and g. Yin et al. [53] indicated that SEM analysis of *Pseudomonas aeruginosa* strain MCCB 102 accumulated heavy metals in the cell wall and along the external cell surfaces. This suggested that heavy metals uptake involves both surface phenomena and diffusion [54].

The plain CA-thin film at low magnification (X35) was presented in Plate 3h. The plain CA-thin film before and after removal of cobalt at magnification (X500) was shown in Plate 3i and j. The CA-thin film immobilized with bacteria and fungi were shown in Plate 3k and m at magnification (X300) to illustrate changes in the surface feature due to

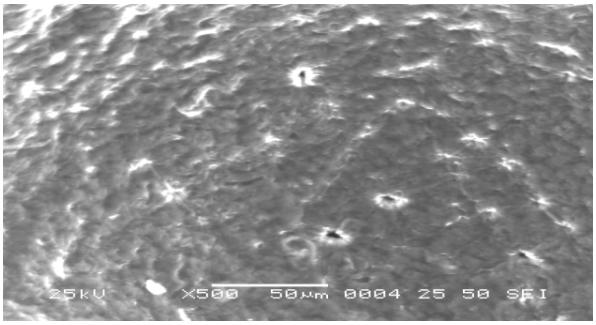


(a)

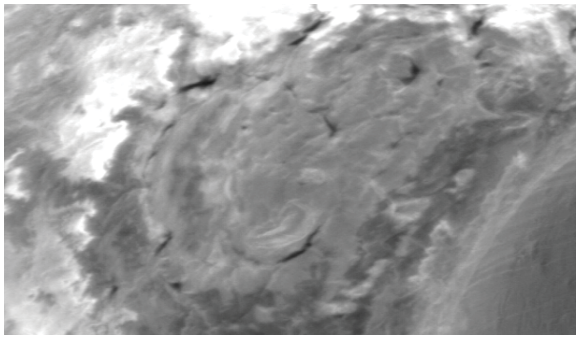
Before Co(II) ions removal **After Co(II) ions removal**



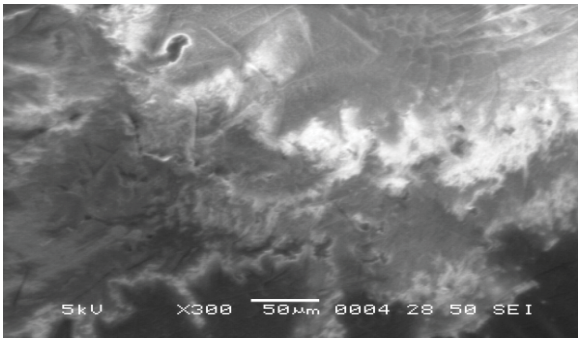
(b)



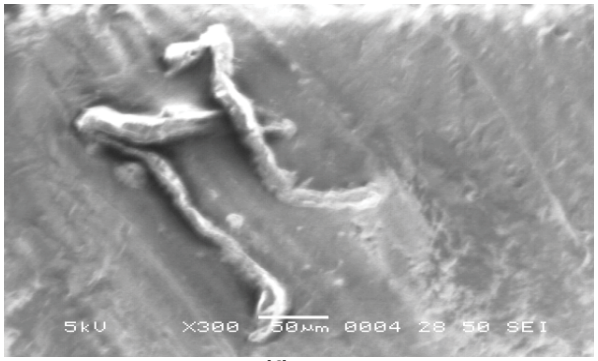
(c)



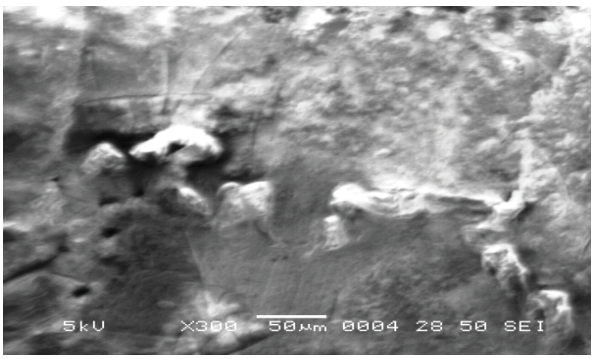
(d)



(e)

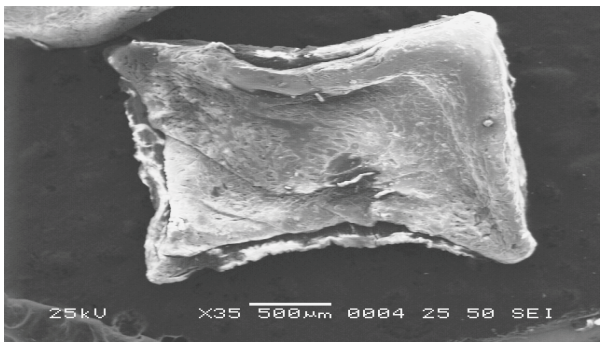


(f)



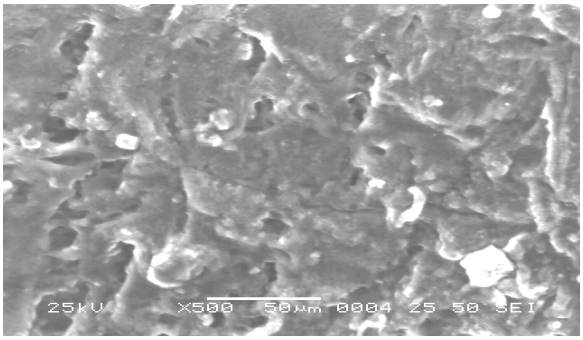
(g)

Plate 2. SEM showing the CA-beads with magnification 50× (a) and the surface feature of different immobilized beads before and after removal of Co(II) ions at 300× (d–g). (a) Control CA-beads before removal of Co(II) ions, (b) control CA-beads, (c) control CA-beads, (d) immobilized bacteria beads, (e) immobilized bacteria beads, (f) immobilized fungi beads, and (g) immobilized fungi beads.

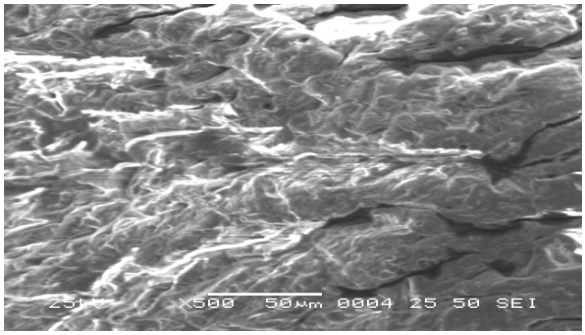


(h)

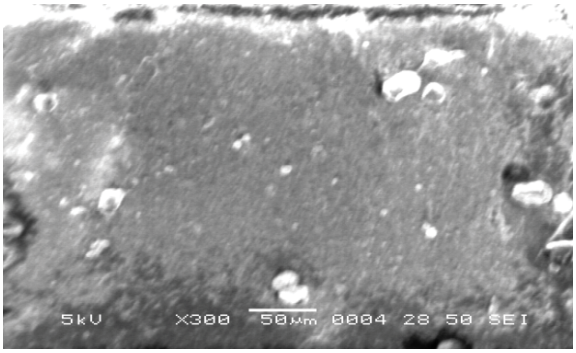
Before Co(II) ions removal **After Co(II) ions removal**



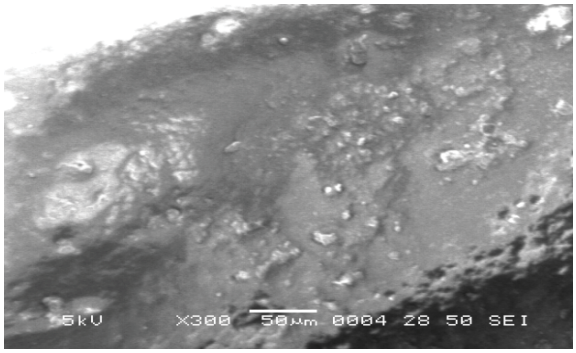
(i)



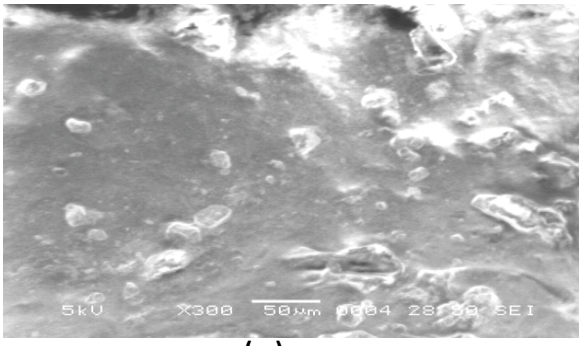
(j)



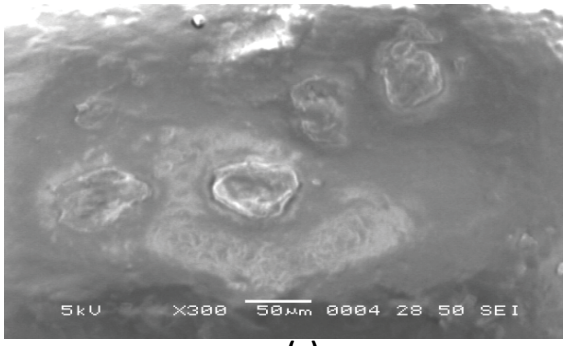
(k)



(l)



(m)



(n)

Plate 3. SEM showing CA-thin film with magnification 35× (h) and the surface feature of different immobilized thin films before and after removal of Co(II) at 300× (k–n). (h) Control CA-thin film before removal of Co(II) ions, (i) control CA-thin film, (j) control CA-thin film, (k) immobilized bacteria thin film, (l) immobilized bacteria thin film, (m) immobilized fungi thin film, and (n) immobilized fungi thin film.

immobilization. Also, the immobilized CA-thin films after the removal of cobalt ions were illustrated in Plate 3l and n.

3.6. EDX analysis

The data of EDX spectra consists of peaks corresponding to all the different elements that are present in the sample. Every element has unique characteristic peaks of energy. Based on EDX elemental analysis the following chemical elements were identified O, Ca, Co. These results confirm the removal and binding of Co as shown in Fig. 8.

3.7. Statistical analysis

Statistical studies were done using Statistical Package of Social Science (SPSS) [55] to compare between types of isolates (bacteria and fungi) and between the isolates of each type in either free or immobilized shapes (beads and thin) for the efficiency of Co(II) ion removal.

The statistical analysis showed significant high mean with (p-value < 0.05) for the removal percentage and capacity of bacteria vs. fungi either free or immobilized in beads and thin-film shapes. The immobilized thin film shape showed a significant high mean than bead shape for the removal percentage in both bacteria and fungi. On the other hand, there was no significant (p-value > 0.05) difference for the removal capacity of immobilized beads between bacteria, and fungi. Also, there was no significant difference in removal percentage and capacity between different bacterial species and fungal species in the free form or immobilized in any used shape.

Studying the effect of environmental conditions on the efficiency of immobilized bacteria and fungi for removal of Co(II) ion, it was found that there was a significant high

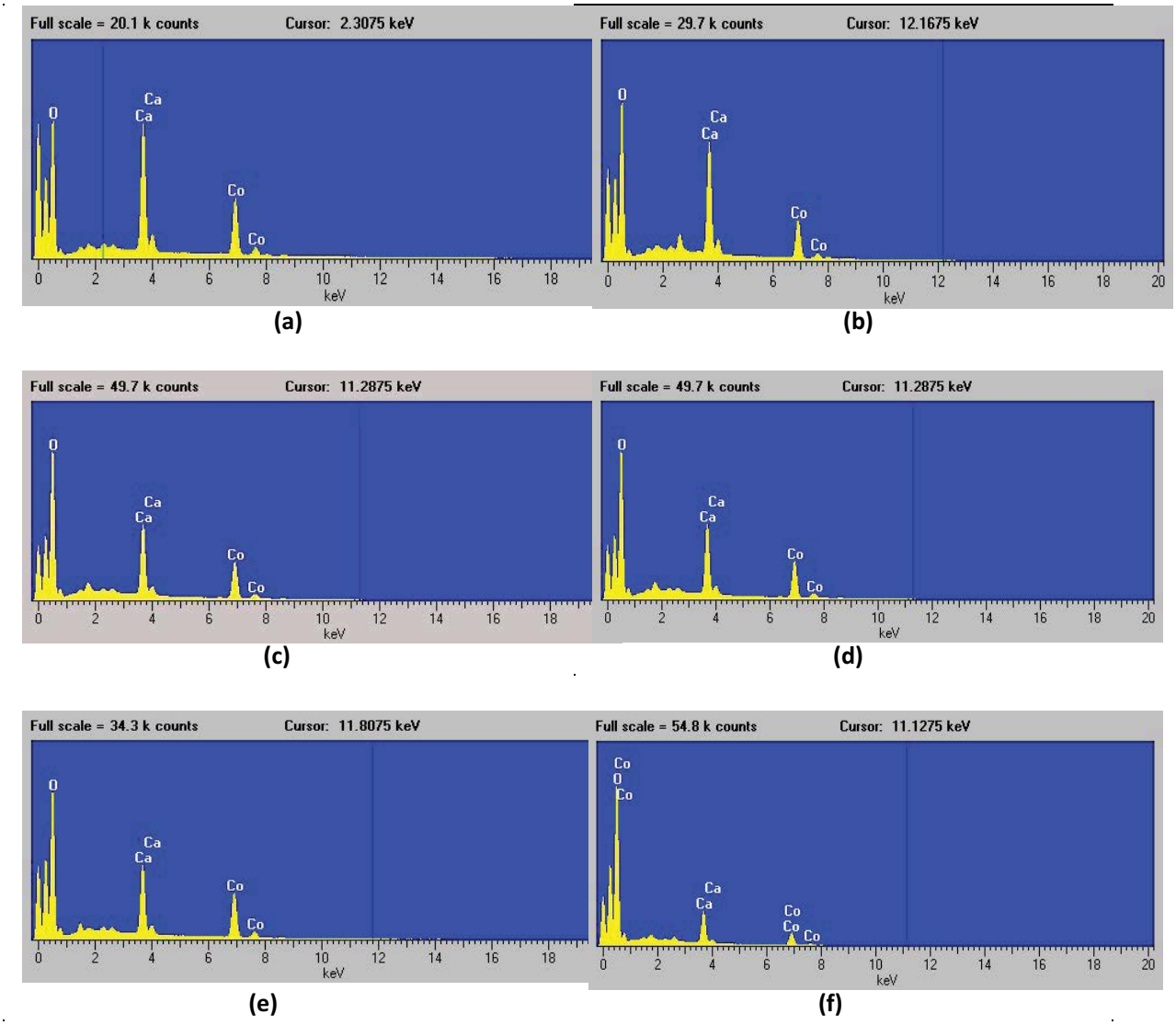


Fig. 8. EDX spectra of CA-immobilized with bacteria and fungi in different shapes after removal of Co(II) ions. (a) Control CA-beads after Co(II) removal, (b) control CA-thin film after Co(II) removal, (c) immobilized bacteria beads after Co(II) removal, (d) immobilized bacteria thin film after Co(II) removal, (e) immobilized fungi beads after Co(II) removal, and (f) immobilized fungi thin film after Co(II) removal.

mean of removal percentage in fungal vs. bacterial when use beads shape. The effect of environmental conditions on the bacterial species showed that there was no significant difference in both removal percent and capacity between bacterial species and when using any shape of gel. The effect of environmental conditions on the fungal species showed that there was a statistically no significant difference in both removal percent and capacity in different fungal species and when using any shape of gel. However, there was a statistically significant high mean for Cobalt-60 removal capacity when use immobilized beads more than using immobilized thin film.

4. Conclusion

The free and immobilized biomass of bacteria showed higher removal capacities with a significant difference than fungal biomass despite having a lower or the same removal percent. This could be attributed to the higher surface area to volume ratio and low dry weight of the bacterial biomasses compared with a dry weight of fungal biomass. Also, results showed that the free dry mycelia had high removal percent and capacity than free wet fungal mycelia. The immobilized thin film shape showed significantly high means than bead shape for Co(II) removal percentage in both bacteria and fungi.

It was clear that the optimum immobilized wet biomass for bacteria and fungi in both beads and thin-film shape was 0.025 g/5 mL sodium alginate gel. Results showed that the optimum pH was 6.5 ± 0.5 and equilibrium was reached after 90 min. The optimum temperature was 25°C for bacteria and for fungi extended to 37°C. The increase in Co(II) ion concentration resulted in a decrease in removal percent and an increase in the removal capacity. The highest removal capacity was obtained at 750 ppm Co(II) ion concentration was 69.4 and 64.96 mg Co(II) ion g^{-1} dry weight of immobilized thin film of *B. haynesii* and an immobilized thin film of dry *A. foetidus*, respectively. The statistical study indicated that the environment conditions had a significant effect on the biomass of when immobilized in the bead shape. The removal capacity of Cobalt-60 by immobilized beads showed statistically significant high mean than immobilized thin film for both bacteria and fungi. The immobilized beads of dry *P. oxalicum* showed higher Cobalt-60 removal percent (79.3%).

SEM for beads and thin films at low and high magnification indicted the changes in surface featured before and after removal of Co(II) ions. The EDX spectra confirm the accumulation of Co(II) ions on the surface of the immobilized biomasses for both beads and thin film.

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