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Cells Immobilization of Some Microorganisms as a Tool for Bioremediation: C-*Pseudomonas putida*

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Abstract. *Pseudomonas putida* was selected for immobilization and heavy metals treatment. Final concentration after treatment for lead by immobilized *Pseudomonas putida* were (9.88, 18.85 and 27.08) ppm respectively. Removal efficiency in current study was: 1.13%, 6% and 10% respectively. The low removal efficiency in current study because the lead (Pb^{+2}) concentration lead to poisoned the bacteria and increase the probability of kill of bacteria and decrease the lead that removed from aqueous solution after treatment by immobilized bacteria according to ASTDR list that explain the lead is lie second position in the ATSDR 2017 Substance Priority List. We are used immobilised *Pseudomonas putida* in treatment the cadmium, the results that record after treatment were (1.88, 3.88 and 9.12) ppm respectively. Removal efficiency for three concentrations was(81,81,70)%.In Present study we used FTIR and SEM technique to explain how the treatment process was occur .

Keyword: Immobilisation, Bacteria, Biotechnology

INTRODUCTION

Immobilisation it is attachment or confirmed of living microorganisms with some compounds to prevent their mobility to produce new properties differ from their other living free but retain their activity and may be reuse them [1].For immobilised organisms a number of benefits other than free living organisms, including product facility isolation, reusing of hormones and enzymes, increase the production quantity, increased operation monitoring, ... etc. [2].

Elements are a part of the habitat & an significant portion of our life. Many elements such as Fe, Al, Cu, Pb, ..etc has been used yearly in USA [3], elements which discharge from sewer to the surrounding in absence of really remediation have turn into a major international worry in present [4] .Bioremediation is an advanced methods to elimination & restoration of elements ions from contaminated zones, & encompasses utilizing microorganisms to minimal dangerous styles, by algal or bacterial or fungal or plantal activities. [5].

Many tests by bacteria (*Pseudomonas spp*., *Enterobacter spp*., *Bacillus spp*., and *Micrococcus spp*.) had been done on many of trace elements, the best absorption/adsorption ability return to the elevated of surface/volume proportions for bacteria [6].

Biosorbent materials found in nature, such as biomass, agricultural wastes, microorganisms, and many others, have been shown to be effective adsorbents for heavy metal ion removal. Because of their high metal binding capability, they are regarded very efficient adsorbents [7].

MATERIAL AND METHODS

Bacterial selection: *Pseudomonas. putida* was used in immobilization experiments . Bushnell-Haas medium was used for isolated bacteria from oil contaminated soil (Table1) [8], and the bacterial were cultured due to the mentioned in [9]. A sample was then taken from each colony after labeled it and loaded in the card then examined and identified by Vitek2 technique. The required culture, which belongs to *P. putida* was isolated. The colonies of the required bacteria (*P. putida*) were transferred to macconkey agar perti dishes for purifying them completely and

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then transferred to the Brain Heart Infusion broth for multiplication and after incubation for a period of 48 hours were ready for the required tests.

TABLE 1. Structure of Bushnell-Haas medium to isolate the dominant bacteria in oil-contaminated soils and its derivatives

Ingredients	**g/l**
$MgSO_4.7H_2O$	0.2
$CaCl_2$	0.02
KH_2PO_4	1
K_2HPO_4	1
NH_4NO_3	1
$FeCl_3$	0.05
Agar	20
Final pH (at 25°C)	7±0.2

Two percent from "sodium alginate" solution was mixed with the same amount of bacteria after filtering 100 ml from the examined bacteria in the quiescent stage with Millipore filter paper 0.45micro m. and shook vigorously to mix the materials, and then poured into a syringe. After this, a second beaker was prepared with $CaCl_2$ (1Molar), and the materials in the syringe were progressively dripped into $CaCl_2$ solution.

Finally, a drop was solidifies and becomes immobilised as of beads after 5-10 minutes and collect these beads and washed with D.W. To see how effective immobilized organisms was in removing lead and cadmium, 10 ml from three standard concentrations 10 mg/l , 20 mg/l , & 30 mg/l was examined .A bioreactor was built as show in Figure.1 and put the prepared volume from each heavy metals in reactor for 15 min. After that, the sample was taken and AAS measurements were taken.

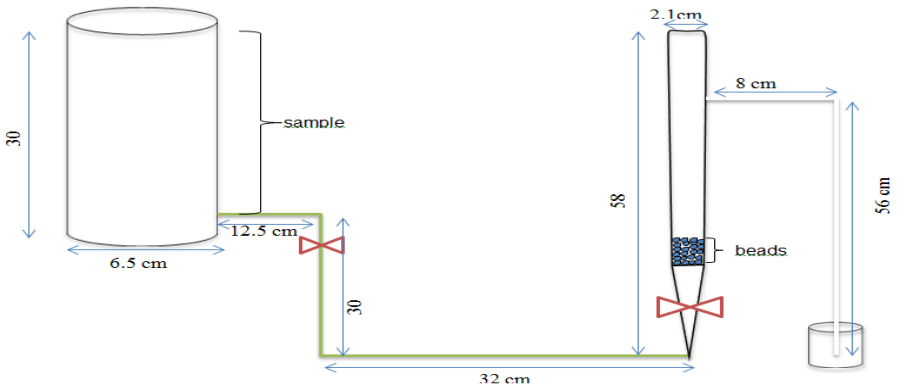


FIGURE 1. Scheme of Device for Microorganisms Immobilisation

RESULTS AND DISCUSSION

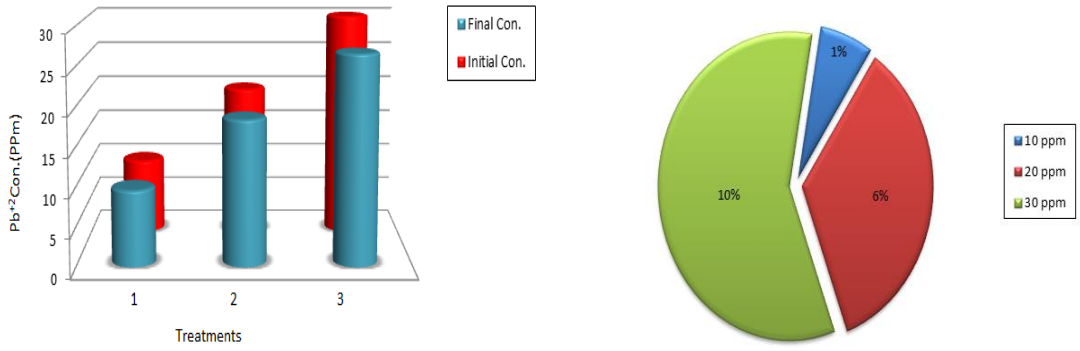
Wastewater treatment techniques, for example, physical and chemical techniques have been broadly used to expel trace elements from polluted sewage. These operations might be incompetent or costly, particularly when the trace elements particles are in media containing in the approximately for 1-100 ppm [10]. Bioremediation techniques, for example, bioadsorption/bioaccumulation for the expulsion of trace elements particles may give appealing substitutional to physical and chemical strategies [11]. From Fig.2a that represents the initial and final concentration before and after treatment for lead ($Pb^{+2)}$ by immobilized *P. putida*. The concentrations of lead were 9.88 ppm, 18.85 ppm, and 27.08 ppm after treatment respectively respectively(Table 2).

TABLE 2. Concentrations of Pb^{+2} and Cd^{+2} before and after treatment for *P.putida* used in current study

	Treatment	**Pb^{+2}/ppm**			**Cd^{+2}/ppm**		
P.putida	Before	10	20	30	10	20	30
	After	9.88	18.85	27.08	1.88	3.88	9.12

Removal efficiency in current study was:1.13% ,6% and 10% for initial concentrations:10 ppm ,20 ppm and 30 ppm as show in Figure 2b.

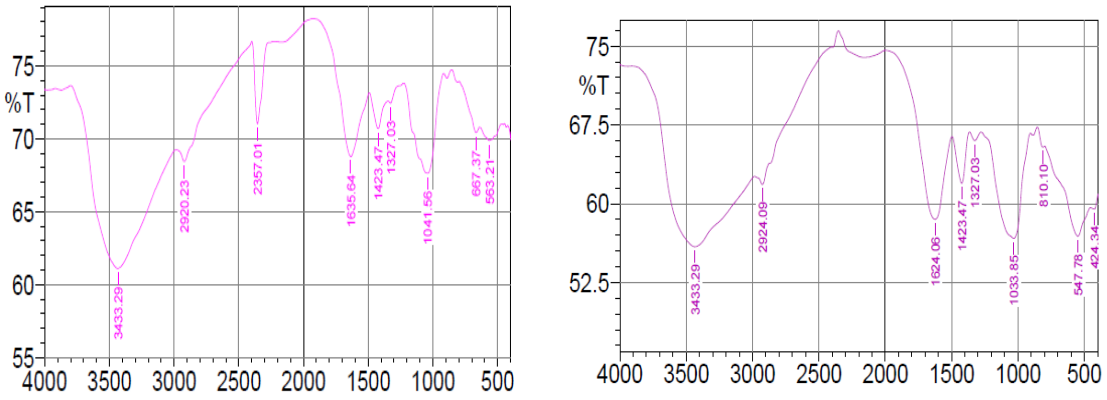
The low removal efficiency in the current study because the lead (Pb^{+2}) concentration lead to poisoned the bacteria and increase the probability of kill of bacteria and decrease the lead that removed from aqueous solution after treatment by immobilized bacteria according to ASTDR list that explain the lead is lie second position in the ATSDR 2017 Substance Priority List, this means that the mechanisms of bioadsorption(Active & Passive) are stopped and only the adsorption on surface is work. [12].



(a) (b)

FIGURE 2. Pb^{+2} Results During the Treatment by *P.putida*, (a): Pb^{+2} Concentrations Before & After Treatment, (B):Removal Efficiency

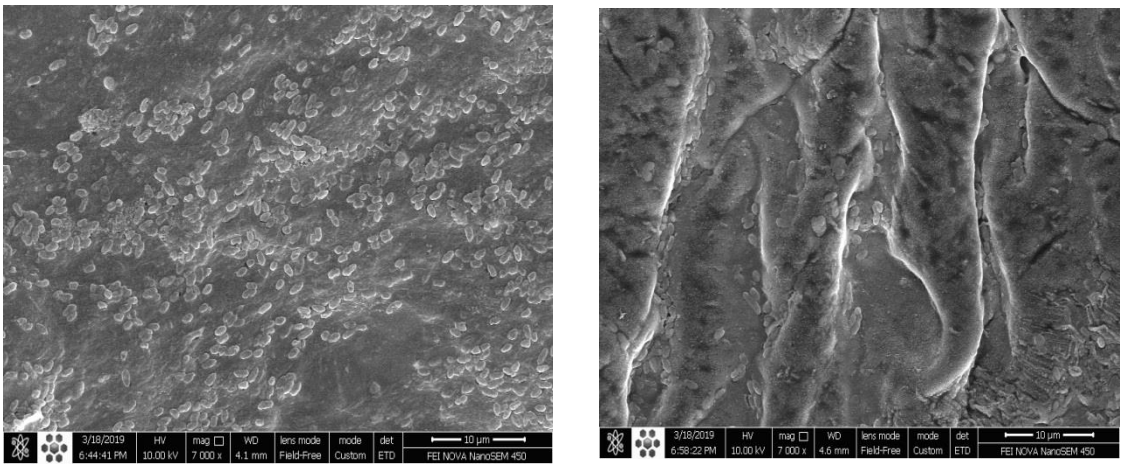
From the peak results of FT-IR in the current study the spectrum of wavelength that ranged from (500-4000)cm-1 and the wavelength that record in free and immobilized Pseudomonas putida were shifting from one site to another because the binding between the functional groups with lead that cause the vibration changes and therefore the new wavelength was appear, FT-IR spectrum is utilized as to affirm accessibility of restricting locales that discovered (O-H) (N-H)C-OP-H(C=O)(CH_2)bonds ,study by [13] who utilized FT-IR spectrum to determine the nearness of amide, carboxyl, and phosphate bunches in some bacterial species Biomass. A few band changes enabled the creators to anticipate the conceivable contribution of amino, carbonyl, carboxyl, and phosphate bunches in the biosorption of some heavy metals which represented in Figure 3.



(a) (b)

FIGURE 3.FT- IR of *P. putida* Used in Pb^{+2} Treatment, (a): After, (b): Before

Scanning Electron Microscope micrographs obviously show in Figure 4, few changes in morphology of bacteria and precipitation of little amount from lead on *P. putida* after treatment developed in lead stacked condition seemed thick, firmly stuffed and with lead statement on outside of bacteria. Trace elements statement on outside of bacteria portrays the bioadsorption marvel of biodegradation. Comparative of bacterial morphology adjustments were seen with different types of bacteria [14]. By trace elements pressure and from current study it was seen that the alteration was outside of bacteria and surface characteristics [15].



(a) (b)

FIGURE 4.FE-SEM of *P. putida* used in Pb^{+2} Treatment, (a): After, (b): Before

Cadmium (Cd^{+2}) represents high poisonous and regular from the trace elements contaminants of effluents. cadmium is reach to the water purification methods such as metal plating industry. Because of the extremely harmful ability of it & it is tendency to bioaccumulation in organisms, releases containing Cd^{+2} are closely monitored [16].

In the present study which used immobilised *P. putida* in treatment the cadmium with three initial concentrations(10,20&30) ppm after treatment the final concentrations were (1.88, 3.88 &9.12) ppm respectively as appear in Figure 5a because of the bioadsorption and bioremediation of immobilized *P. putida* for cadmium.(Table 2).

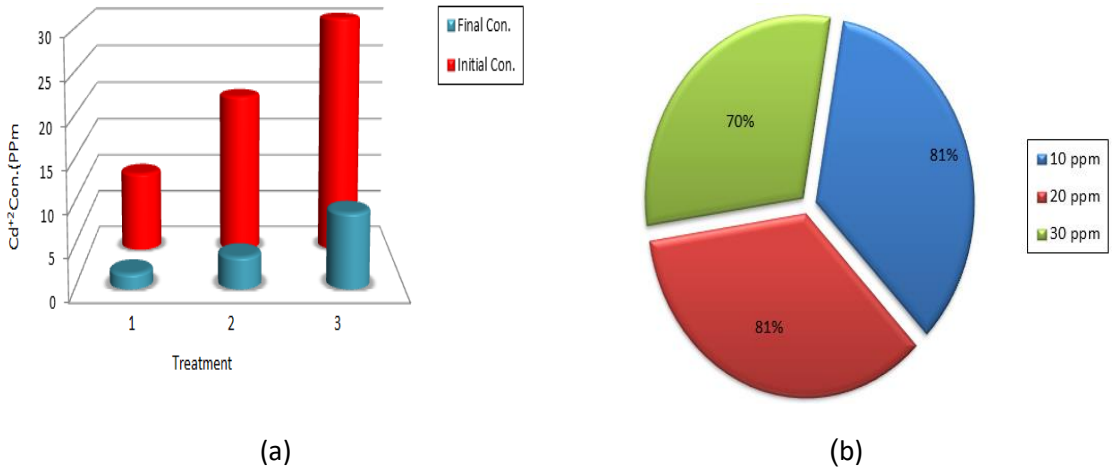


FIGURE 5. Cd^{+2} Results During the Treatment by *P.putida*, (a): Cd^{+2} Concentrations Before & After Treatment, (b): Removal Efficiency

Utilization of microorganism or its biomass or its outputs for the recuperation of elements from sewage and the bioremediation is the financially savvy and ecological well-disposed procedure, which has been broadly acknowledged by bacterial elements rebioabsorption [17]. Removal efficiency for three concentrations was(81,81and70)% respectively(Table 3), these are because they have been demonstrated that the kindship of cationic species for the useful gatherings present in the surface of cell and therefore lead to binding with negative charge on surface of cell caused the remove of cadmium from aqueous solutions [18].The high removal efficiency in contrast with the lead return to the resistance ability of *P. putida* for Cadmium, the high removal efficiency at 10ppm and 20ppm and decrease at 30ppm because the strained impacts of cadmium expanded as the level of this component was raised in growth media and when nearest to 40 mg/l Cadmium caused demise in a significant number of the bacteria[19].Figure 5b. Current result of FTIR which showed in Figure 6 explain the distinctive tips of (O-H), (N-H) &(C=O=C) gatherings showing up in FT-IR examination affirmed the nearness of these moiety of bacteria & the shift in "pinnacles" which utilized to address the authoritative of Cadmium with bacteria and the move in these tips and transmittance level was because of the connection of Cd^{+2} with carbonyl mediate. Expansion to this, the rate transmittance of tops in FTIR spectra of bacteria that treated with cadmium, was impressively less noteworthy than those of control bacteria. This demonstrated extending of securities, jumped out at lower degree (because of occur of Cd^{+2}), came about to lessen their rate transmittance as detailed by [20].

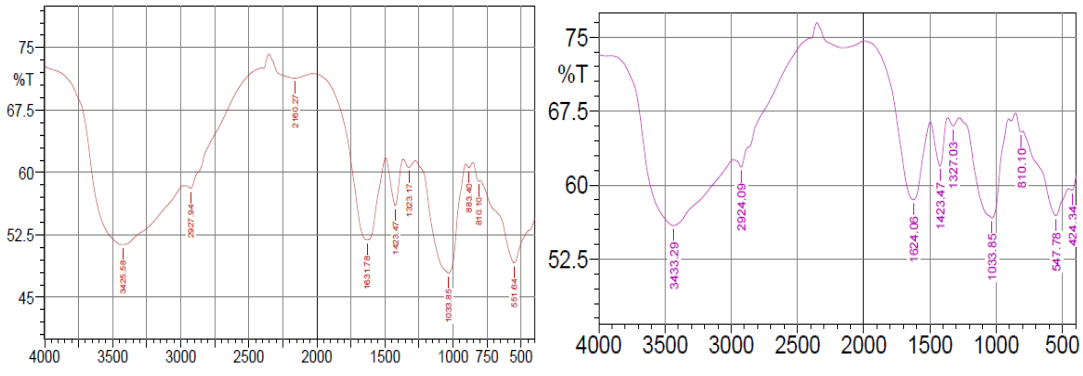
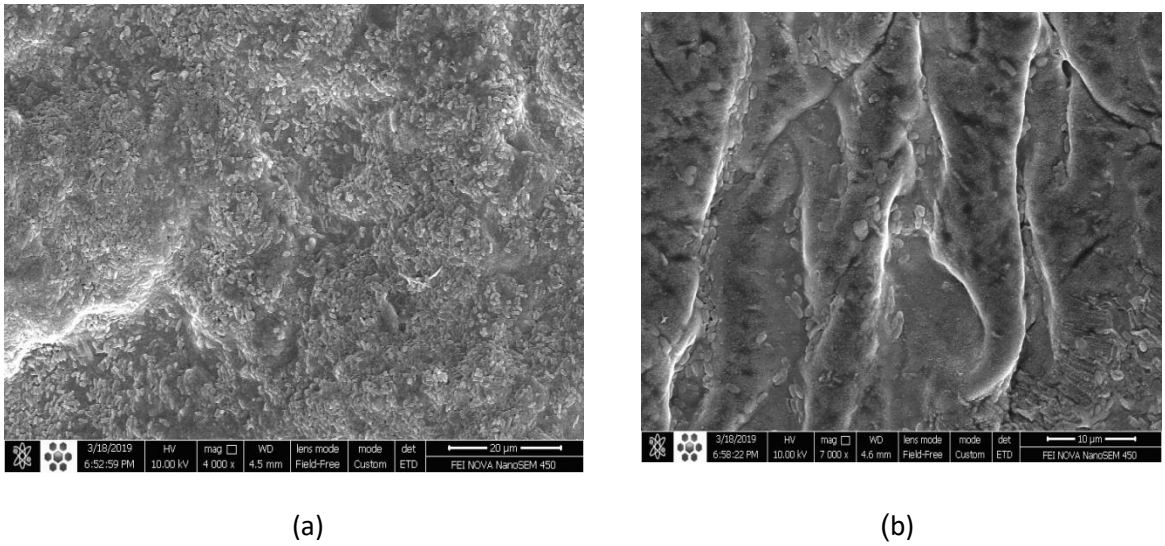


FIGURE 6.FT- IR of *P. putida* Used in Cd^{+2} Treatment, (a): After, (b): Before

From SEM micrograph results in Figure 7 appears the adsorption of Cadmium at the surface of *P. putida* when we compare between the micrograph of *P. putida* before and after treatment and the portrayal of bioadsorbents by SEM offers morphological and basic data the rigid to the huge profundity for area, permitting diverse example portions of remain with core interest. Scanning electron microscopy additionally has high goals and higher amplification is feasible for firmly separated materials. Notwithstanding its ability to create clear picture, it is valuable in demonstrating the morphological subtleties of bioadsorbents. In any case, scanning electron microscope has impediments on its most minimal recognizable molecule size and its powerlessness to identify follow components in a matter [21] [22] [23].



(a) (b)

FIGURE 7.FE-SEM of *P. putida* Used in Cd^{+2} Treatment, (a): After, (b): Before

CONCLUSION

Immobilized organisms are more effective for wastewater treatment, Removal efficiency is increased by increased the initial concentration, and immobilization is more effective for cadmium removal than lead. Carboxylic functional groups are good bind with heavy metals in contrast with amide and carbonyl groups. FESEM and FTIR are beneficial Techniques for detection the wastewater pollutants removal.

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