# Enhancement of Survival and Uranium Bioleaching and Bio-Sorption Properties of Bacteria by Immobilization Techniques in Egypt

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## ABSTRACT

The Egyptian bacterial strain E. coli was isolated from Uranium Ore and used for bioremediation studies. Capsulated in calcium alginate beads, A Burette-flow- packed bed made of these beads was used for bio-sorption of U by rate of 968 ppm U m-3 water per day with a sorption efficiency of 93.2% was achieved with studying optimum condition for bacterial growth and bio-sorption process and bioleaching processes. Various isotherm models of adsorption such as Langmuir, Freundlich, were studied. The results predicted adsorption in a multi- layered via physio-sorption. In this study 6 bacterial isolate, isolated from Uranium Ore, from Aborshid Egypt, were characterized for their response to 15 antibiotics and 10 heavy metals beside uranium. Multiple resistant isolates were observed, the results revealed a varying response of the Ore bacteria to the tested U & heavy metals. All isolates demonstrated numerous metal protections towards 10 heavy metals, with MIC ranging from 50 to 1000 ppm. The strongest of the strains in both groups were resistant to Pb, Ni, Cu and Zn. Highly metal-resistant bacteria could be used with potential application for treatment of wastewater using immobilized bacterial cells, the most potent isolate was identified. The Egyptian strains belong to E. coli, based on 16S rRNA gene sequencing the nucleotide sequences reported here were deposited to the NCBI Nucleotide Sequence Database under accession numbers (MF496270) by the name of MostafaGomaaFadl this result confirmed by scan (EDX) FT-IR ICP device. Potent metal bio-sorbents 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 increment bio-sorption onto the cell surface. Starting with Isolation and identification of heavy metal-resistant bacteria from rock Ore. Studying Factors Affecting Uranium Bio-sorption, Optimization of bacterial growth conditions and optimum for metal uptake by free and immobilized bacterial cells and Desorption ratio of uranium ions adsorbed by E. coli /alginate, 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 bio-sorption 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 bio-sorption.

Keywords: wastewater, E. coli; Bioleaching; Ca-alginate, Capsule; Uranium, Bio-sorption

### **INTRODUCTION**

#### Metal recovery by microorganisms

The metal can be Uptake by the microorganisms through two processes: bioleaching and biosorption. Ore microorganisms are very closely involved and play important role in many geological processes. These include mineral formation, mineral degradation, precipitation and mineral recycling. In recent years, a new trend of mineral science deals with application of biotechnology in mining industry. Practically, microorganisms can be successfully used for solubility of metals (e.g., copper, zinc, cobalt, lead, uranium) from low-grade ores. Mining with microbes is both economic and environmental safety [1]. To getting rid of that solute by general physical separation techniques is a sharp target and chemical treatment is not always ecologically favored. In present study, hyper saline dissimulators autotrophic bacteria found to remove heavy metal including lead metals from saline waters [2,3].

#### Enhancement of microbial biomass as bio-sorbents

Several techniques used leading to improvement of Uranium and heavy metal bio-sorption efficiency incorporate the advancement of more powerful bio-sorbents and the outline of more efficient bio- sorption processes. Bio-sorbent development may be reached by either isolating organisms with high accumulation level or high liking to Uranium and heavy metals although it

has been proposed that microbial biomass may be used as a bio-sorbent for metal up-take from industrial waste pole and hence assume a noteworthy part in bioremediation, it's down to earth use in the local frame presents issues its practical use in the native form does present problems [4]. Most forms of microbial biomass are either very fine suspensions (yeast, bacteria) or Mycelium materials (filamentous fungi) and as such are not compatible with use in continuousflow systems. The resulting bio- sorbents are either difficult to separate from the sorbate or result in reduced flow rate in continuous-flow system.

#### Entrapment of bacteria by capsulation like technique

Enhancement of bacterial biomass as bio-sorbents by increase its capacity by immobilization, Where the high bio-sorption yield obtained by bacteria, the uranium and heavy metal bioremediation process expects microorganisms to be joined to a strong surface. Surface obsession and cell ensnarement are the two strategies forimmobilization. Different materials were tried for cell immobilization [5,6]. Support materials suitable for biomass entrapment include alginate, silica gel, cellulose and glutaraldehyde [7-9]. The polymeric matrix provides the mechanical and chemical stress of the last bio- sorbent molecule to be under go for repetitive sorption-desorption cycles, so it is important to choose the correct fixation matrix. Akar measured the bio-sorption of 100 mgL<sup>-1</sup> of nickel at pH 6.5 to be 33.83 and 7.50 gg<sup>-1</sup> for silica gel and Proteus vulgaris, respectively, whereas the immobilized bio-sorbent had a bio-sorption limit of 45.48 mg<sup>-1</sup> under the same conditions [10].

Maximum most extreme bio-sorption acquired utilizing immobilized biomass gives guarantee to immobilize cells in a section reactor for the remediation of substantial metals. At pH 5.0, the Cd<sup>2+</sup> bio-sorption capacity of E. coli biomass-free beads was 1.30 mg<sup>-1</sup>, which was significantly lower than the bio-sorption efficiency of immobilized whole cells, showing a capacity of 2.18 and 4.41 mg/g for biomass stacking of 8.42 and 19.5 wt.%, respectively [11]. The removal efficiencies of both Cu and Cd for Ca-alginate beads were found to increase to 95% within 3 hours in the temperature range of 15-40°C, indicating that it is possible to treat waste using Ca-alginate beads within a short time period under various field conditions [12-16]. Despite the fact that calcium alginate is helpful for entangling cells in its gel structure, its leverage dwells for the most part in the re-use of the captured cells. Nevertheless, the high overwhelming metal fondness of alginate makes it unusable for the improvement of consistent modern procedures, as the recuperation of the alginic corrosive would increase the final costs of effluent treatment.

Successful bacterial immobilization was accomplished on latent surfaces, for example, teflon layers, silicone elastic and polyurethane froths. Best after effects of surface obsession were got with Pseudomonas veronii, which could develop on each of the three surfaces. This organism developed a film over the matrix surfaces and also formed aggregates and adhered to glass during column culture work. The development of other bacteria on the same surfaces was barely observed on E. coli is very effective bio sorbents for heavy metal removal [17-20]. In addition, several other metal-binding proteins, such as metallothioneins (MTs), phytochelatins (PCs) used recombinant E. coli bio-sorbents with overexpression of MerP proteins for the bio-sorption of Cu, Ni, and Zn from aqueous solutions [21-23]. Deng demonstrated bio- sorption by immobilized recombinant cells expressing human metallothionein proteins [24]. Samuelson created recombinant staphylococcus species strains with surface uncovered fanciful proteins containing polyhistidyl peptides [25]. The two strains of staphylococci increased enhanced nickel-restricting limits because of the presentation of peptides into their surface proteins.

As the bio-sorption process is engaged with primarily cell surface sequestration, alteration of the cell divider can extraordinarily adjust the official of metal particles. A number of methods have been employed for cell wall modification of microbial cells in order to enhance the metal-binding capacity of biomass and to elucidate the mechanism of bio-sorption. Physical treatments include heating/ boiling, freezing/thawing, drying and lyophilization. Utilized for biomass alteration incorporate washing biomass with cleansers, cross connecting with natural solvents and antacid or corrosive treatment. Pretreatments could adjust the surface qualities/bunches either by evacuating or concealing the gatherings or by uncovering more metal- restricting destinations [8,9]. Immobilized individual enzymes can be successfully used for single-step reactions. However, they are not suitable for multi-enzyme reactions and for the reactions requiring cofactors. The whole cells or cellular organelles can be immobilized to serve as multi-enzyme systems. In addition, immobilized cells rather than enzymes are sometimes preferred even for single reactions, due to cofactor in isolating enzymes. For the enzymes which 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 that 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.

Immobilized dead cells: In many instances, immobilized non-viable cells are preferred over the enzymes or even the 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.

#### AIM OF THE STUDY

#### **Objectives**

The following hypothesis will be tested during this research:

Biochemical characterization of bio-sorption bacteria will identify candidates suitable for evaluation of bio-sorption encapsulation technology.

Bioleaching by immobilization of bacteria using pea protein-alginate beads will enhance bioleaching and accumulation rate.

#### Applications

The uranium recovery from phosphoric acid and laboratory waste: Phosphorus is a fundamental plant supplement and is taken up by plant roots, usually gotten from phosphoric acid,  $H_3PO_4$ . Generally, the phosphate rocks contain an appreciable amount of uranium in the range of 0.005-0.03%  $U_3O_8$  mainly [25,26]. The uranium recuperation from phosphate deposits is dependable where its concentration varies from one locality to another [27,28]. The uranium contents in the Egyptian phosphates range is more or less, between 40 and 100 ppm and in some types reaches 200 ppm [29,30]. Most of the uranium content in the phosphate ores transfers into the phosphoric acid and the phosphate fertilizers during their production from the raw material. Situation like that may cause a genuine ecological pollution on the long run due to accumulation of the uranium in the soil and its probable transferring to the water bodies produce serious toxicological concerns such as cancer, kidney and liver injuries [31].

Bio-sorption course has been discovered to be better than different advances in light of its high effectiveness, helpful operation, low cost, regeneration of bio-sorbents and recovery of metals, compared to the traditional effluent treatments [2,3]. It was evident from a literature survey of >100 recent papers that low-cost adsorbents had demonstrated outstanding removal capabilities

for various pollutants [32]. Bio-sorption utilizes all kinds of microorganisms including yeast, bacteria, algae, molds and protozoa, which could be discovered everywhere. The mechanisms for removal of heavy metals include adsorption, uptake and reduction of methylation and oxidation [33]. Living, dead and immobilized bacteria could be used in the process. Immobilized bacteria are normally simpler to deal with require less overwhelming detachment frameworks, enable a high biomass thickness to be kept up and give a more prominent opportunity for reuse and recovery [34].

Bio-sorption study were conducted by the administration of E. coli or alginate-chitosan microcapsule to uranium polluted phosphoric acid and the significant bio-sorption ability was evaluated in order to remove uranium ion from aqueous solution using free E. coli as the control.

The contamination of nature with dangerous overwhelming metals is spreading through the world alongside industrials progress. Cadmium, lead and other substantial metals are of significant enthusiasm because of their lethality and broad application in the industry [35]. Along these lines, it is important to create touchy, compelling and reasonable strategies, which can proficiently screen and decide the nearness and measure of perilous metals.

Traditionally, in the environment natural hazard caused by overwhelming metals contamination is controlled by evaluation of aggregate metals after assimilation with solid acids by using conventional analytical methods [36]. Conventional techniques to analyze metals include chemical precipitation, ion exchange, chelation, membrane separation, cold vapor atomic adsorption spectrometry, inductively coupled plasma mass spectrometry, UV visible spectrometry and X-ray absorption spectroscopy. These techniques are highly precise though suffering from disadvantages of high costs.

Therefore, improvement of a simple alternate system for monitoring substantial metals by microorganisms, due to its potential applications in environmental protection and lethal substantial metals removal is fundamental. Biosensor is an explanatory gadget that comprises of immobilized organic material in hint contact with a good transducer, which will change the biochemical signal into a quantifiable electrical signal.

Bacterial biomass bio-sorbents: Mann reported that bacteria microscopic organisms are the most inexhaustible and flexible of microorganisms and constitute a huge division of the whole living earthly biomass, whose mass is estimated as ~1018 g. In the mid-1980s, certain microorganisms were found to amass metallic components at a high limit was known as bacterial bio-sorption

[8,9].

Due to their little size and capacity to become under controlled conditions, and their accommodation to an extensive variety of ecological situations. Furthermore, inexpensive nutrient sources are readily available for microbes. Potent metal bio-sorbents among microorganisms incorporate genera Bacillus, Pseudomonas, Streptomyces, Micrococcus and Escherichia coli. Bio-sorbents got from bacterial biomass have since turned out to be common. The limit of bio-sorption not just relies upon the sort of metal particles, yet in addition of bacterial variety because of varieties in cellular components. Metal particles in solution are adsorbed on bacterial surfaces through interactions with chemical functional gatherings for example carboxylate, amine, amide, imidazole, phosphate, thioether, hydroxyl and other utilitarian gatherings found in cell divider biopolymers. The fast energy seen with bacterial biomass speaks to a favorable viewpoint for the plan of wastewater treatment frameworks. Biosorption incorporates a blend of a few components for example, charge fascination, composition, particle trade, covalent authoritative, van der Waal's forces, adsorption and micro-precipitation. Very short contact times are for the most part adequate to accomplish a metal- bacterial biomass enduring state. This is because biomass is used in the form of either fine pellets or wet viable cells, where mass transfer resistances are usually negligible.

Bacterial cell constituent: The diameter of typical bacterial cells ranges from 0.5 to 1.0 mm; however, some are more extensive than 50 mm). Despite, the fact that there is an incredible assortment of shapes because of contrasts in hereditary qualities and environment microorganisms have basic morphology. The most widely recognized microscopic organisms are available in three essential shapes: circular or ovoid (coccus), bar (bacillus, with a round and hollow shape) and winding (spirillum). The little size of microscopic organisms guarantees quick metabolic procedures. A bacterial cell (E. coli) contains a cell divider, cell film and the cytoplasmic network which comprises of a few constituents that are not layer enclosed (inclusion bodies, ribosomes, and the nucleoid with its genetic material). Bacteria are classified as either gram-positive or Gram-negative are classified by the gram staining microbes into two principal group cell wall qualities [36]. This grouping partitions, (both cell divider sorts contain a peptidoglycan layer that is rich in carboxylate gatherings and totally encompasses the phone [37-39]. Cell divider is a general negative charge, because of the nearness of phosphodiester bonds between teichoic acid monomers.

The exceptionally charged nature of lipopolysaccharides presents a general negative charge on the gram-negative cell divider. The anionic useful gatherings show in the peptidoglycan, teichoic acids and teichuronic acids of gram-positive microscopic organisms and the peptidoglycan, phospholipids, and lipopolysaccharides of gram- negative microbes are the segments fundamentally in charge of the anionic character and metal-restricting capacity of the cell divider [40]. The peptidoglycan layer in the gram-positive cell divider is ca. 2.5 nm thick, while the gram-negative peptidoglycan layer is much thinner (ca. 7.5 nm). The walls of gram-positive bacteria consist of three primary components: cytoplasm mixed with peptidoglycan, to which teichoic acids are covalently bound. The envelope of gram-negative bacteria is more complex than that of gram-positive bacteria. It consists of two membrane bilayers (the outer and plasma membrane) that are chemically and functionally distinct from one another and sandwich a thin peptidoglycan layer between them. Teichoic acids enable the gram-positive [41]. Extracellular polysaccharides (EPSs) provide ability of binding metals. However, their availability rely on the bacterial species and growth conditions, what's more, they can without much of a stretch be expelled by basic mechanical disturbance or concoction washing [42].

The cell dividers of bacteria contain a large number of surface functional groups, in which carboxyl is generally the most acidic group in the bacteria. 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 increment bio-sorption onto the cell surface. A few microscopic organisms have extraordinary structures for example, flagella and the S-layer. Sumin explained that the inner and outer void spaces of the Ca alginate spheriouls were filled during the bio-sorption process with heavy metals such as Cu, Fe, and S, suggesting that heavy metal removal by Ca alginate beads occurs by not only ion exchange but also by framing edifices and precipitation [11].

The S-layer action in bio-sorption: The S-layer action is a surface and para crystalline envelope present in several gatherings of microbes and archaea. This layer is formed from protein or glycoprotein monomers that can self-collect in two-dimensional structures [43]. S- layers are related with lipopolysaccharides [44,45]. Porosity is between 30 and 70% and the diameter of the pore between 2 and 8 nm. This characteristic can be used for metal binding. An imperative normal for this protein is its ability to reassemble once disconnected from the cell [46]. Due to

this effect, it can be used for bioremediation. S-layer proteins may execute a catching part of metallic particles in both living and dead cells, being a potential option for bioremediation of substantial metals in the field. Some bacterial cells can produce a capsule outside the bacterial cell wall.

They are much hydrated and approximately organized polymers cases are made out of starches and proteins of polysaccharides and a couple of comprise of proteins or polymers of amino acids called polypeptides. Container course of action is imperative to metal official [47-50]. EPSs have a high sub-atomic weight with a wealth of adversely charged practical gatherings (ligands), e.g., carboxyl, hydroxyl, and uronic acids [51,52]. Where the arrangement of bacterial EPS is perplexing, contingent upon the strain and its way of life conditions. EPS amalgamation is likewise announced for a few Pseudomonads, Zoogloearamigera, Rhizobium, Klebsiella and Bacillus species. Ordinary constituents of EPS are for the most part polysaccharides and proteins, frequently joined by nucleic acids, lipids or humic substances [53,54].

Uranium sequestering mechanism (bio-sorption): Research is in progress to establish biosorption as a financially reasonable strategy to trap and accumulate metals. Bio-sorption 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) [55]. Adsorption and micro-precipitation involve binding of electrically neutral metals without the arrival of a stoichiometric amount of previously bound ions. In precipitation reactions, the main impetus is interaction between the solute and the solvent, whereas in adsorption affinity amongst sorbent and sorbate is the driving force. On account of physicochemical, the mechanism by which interaction 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 indicate that the bio-sorption of lead and copper by Bacillus sp. involve a particle- exchange mechanism. Since the main mechanism involved in bio- sorption is ion exchange, protons compete with metal captions for the binding sites and for this reason; pH is the operational condition, which influences the process most strongly [56].

#### Factors affecting uranium bio-sorption

There are many factors affecting the bio-sorption process of uranium metal ions by the microorganisms. Some of these components were recognized from the examinations of the bio-sorption procedure, for example particle fixation, biomass concentration, time, pH and

temperature, while other factors belong to types of biomass such as living or dead, free or immobilized cells and the bio sorptive capacity of cell biomass. Kerkar et al. stated that the contact time where the obstruction between restricting locales because of expanded biomass dosages cannot be overruled, as this will result in low specific uptake Which played a vital part in the evacuation of  $Zn^2$  as initial solute concentration appears to have an effect on bio-sorption, with a higher concentration resulting in a high solute uptake [57-60]. In our study uranium bio-sorption capacity increase with increasing contact time. Due to as time passed the vacant sites of bio-sorbent available for adsorption were occupied by the pollutant. While the initial concentration is the significant independent parameter associated with chrome study of Mishra and Tadepalli in uranium study increase in initial concentration associated with increase in bio-sorption percentage of ion removal toxic pollutant.

Therefore, initially the effluent concentration was very less and then started to increase up to 30 h and then decreased. The important independent parameter for  $Pb^2$  and U removal is temperature where optimum temperature for growth of bacteria ranged from 30-40°C. However, pH value affects strongly in the removal of  $Zn^2$ ,  $Pb^2$  and  $Cr^3$  as Mishra and Tadepalli stated in his study also in our study it has significant effect in uranium removal. Bio-sorption by living cells is temperature dependent, hence change in this parameter will strongly affect while the bio-sorption processes by nonliving biomass is not significantly affected by the temperature [57,61].

Freundlich isotherm: This isotherm is an empirical equation and the most widely used isotherm for the description of adsorption equilibrium. It depicts the adsorption of natural and inorganic mixes on a wide assortment of adsorbents including bio-sorbents. qe is the amount adsorbed, K F the characteristic constant related to the adsorption capacity, Ce the equilibrium concentration and the characteristic consistent identified with adsorption power or level of idealness of adsorption.

The plot of Log qe versus log Ce has a slope with the value of 1/ and an intercept magnitude of log K F. Log K F is equivalent to log qe when Ce equals unity. However, in other cases when 1/=1, the value of K F depends on the units upon which qe and Ce are expressed. A Freundlich constant between 1 and 10 indicates favorable adsorption. A bigger estimation of n (littler estimation of 1/n) suggests more grounded association amongst bio-sorbent and overwhelming metal while 1/n equivalent to 1 shows direct adsorption prompting indistinguishable adsorption energies for all destinations [62]. The Freundlich isotherm has the ability to fit nearly all

experimental adsorption-desorption data, and is excellent for fitting data from highly heterogeneous sorbent systems. 1/n esteem higher than solidarity (n<1) proposes the nearness of a bended upward isotherm, infrequently named as a dissolvable fondness sort isotherm [63-67]. Inside this sort of isotherm, the negligible sorption vitality increments with expanding surface fixation. Sorption of solute on any sorbent can happen either by physical holding, particle trade, complexation, chelation or through a mix of these associations. In the mechanism of adsorption will not be restricted to physical bonding. The Freundlich condition cannot foresee the adsorption balance information at extraordinary focuses [68-73].

Moreover, this condition is not lessened to straight adsorption articulation at very low concentrations. However, researchers rarely face this problem as moderate concentrations are frequently used in most bio-sorption studies [73-77]. Langmuir isotherm: The Langmuir where qe is the amount adsorbed, Ce the equilibrium concentration, q max the saturated monolayer adsorption capacity, and K L the sorption equilibrium constant. This equation is often written in different linear forms [78]. In bio-sorption process, the saturation limit of certain biomass is influenced by a few factors for example, the quantity of destinations in the bio-sorbent material, openness of the locales, compound condition of the locales (i.e., accessibility) and partiality amongst site and metal (i.e., binding strength). Because of covalent metal official, assuming that a possessed site is hypothetically accessible, the degree to which the site is to be involved by a given metal depends further on its binding strength and concentration when compared with the metals as of now involving the site. [78-81].

Amid physical adsorption, the holding between substantial metals and dynamic locales of the bio-sorbent debilitates at higher temperatures as opposed to chemisorption holding, which ends up noticeably more grounded [63,78]. The exothermicity or endothermicity of the bio- sorption procedure can be resolved via the heat of adsorption. This thermodynamic property is commonly obtained through an integrated Van't Hoff equation, which relates the Langmuir constant, K L to the temperature, where Ko is the adsorption equilibrium constant, Ea the activation energy of adsorption/heat of adsorption, R the gas constant (0.0083 kJ/(mol K)), and T the absolute temperature (K) [82-88].

Kinetics of heavy metal bio-sorption: Kinetic models have been used to test the experimental data. In addition, information on the kinetics of metal uptake is required to select the optimum conditions for full-scale batch metal removal processes [73]. Adsorption energy is communicated

as the solute evacuation rate that controls the living arrangement time of the sorbate in the solidarrangement interface. A few adsorption motor models have been portrayed for the adsorption energy and rate-restricting advance. These incorporate pseudo-first and second-arrange rate models, the Weber and Morris sorption active model, the Adam-Bohart-Thomas relation the first-order reversible reaction model, the external mass transfer mode, the first-order equation of Bhattacharya and Venkobachar and Elovich's model and Ritchie's equation. The pseudo-first and second- kinetic models are the most widely used models for bio-sorption kinetics of heavy metals and quantify the level of uptake in bio-sorption kinetics [89-91].

Pseudo-first-order kinetics: The Lagergren first-arrange rate articulation in view of strong limit is for the most part communicated as takes after: where q is the sum adsorbed at time t and k1 the rate steady of first-order adsorption. Hypothetically to ascertain the rate constants and equilibrium metal uptake the straight-line plots of log (qe- q) against t were made at various starting metal concentrations [92]. The qe value acquired by this method is then compared with the experimental value. On the off chance that expansive errors are represented, the response cannot be delegated first-arrange in spite of the fact that this plot has a high connection coefficient from the fitting procedure. Nonlinear fitting is a different way to achieve the predicted value of qe and k1. Although this is not a common exercise. The trend shows that the predicted qe values seem to be lower than the experimental values. A time lag, probably caused by presence of a boundary layer or external resistance controlling the beginning of the sorption process, was argued to be the responsible factor behind the discrepancy [93].

Pseudo-second-order kinetics: In that capacity, in contrast with pseudo-first-arrange energy, this model is viewed as more fitting to speak to the dynamic information in bio-sorption frameworks. The pseudo-second-order model is derived on the basis of the sorption capacity of the solid phase expressed as: where k2is the rate constant for pseudo-second-order model. The pseudo-second-order rate constants were determined experimentally by plotting t/q against t. Ho conducted an evaluation using linear and nonlinear methods to Determine the pseudo-second-order kinetic parameters. He chose cadmium as the heavy metal and tree fern as the bio-sorbent. The pseudo-second-order model as written has the highest coefficient of determination.

In contrast to the linear model, the resulting kinetic parameters from the nonlinear model were almost identical among each other. To that end, the nonlinear method is considered as a better way to ascertain the desired parameters. Still most of the bio-sorption studies in the literatures utilize. This tendency comes as an indication that the rate-limiting step in bio-sorption of heavy metals are chemisorption involving valence forces through the sharing or exchange of electrons between sorbent and sorbatecomplexation, coordination and/or chelation [94-105].

Desorption of heavy metals: Bio-sorption is a process of treating pollutant-bearing solutions to render it contaminant-free. However, it is also necessary to be able to regenerate the bio-sorbent. This is possible only with the aid of appropriate elutants which usually results in a concentrated pollutant solution. Therefore, the overall achievement of a bio-sorption process is to concentrate the solute.

Comparison of modified bioploymeric beads with reported bio remedial techniques: Bacillus sp. was isolated rock sample soil at CLC Tannery Complex (22.567°N 88.367°E, Bantala, Kolkata, West Bengal, India) and was observed to be exceptionally U (VI) safe [Minimum Inhibitory Fixation (MIC) of U(VI) against Bacillus was 4.2 g<sup>-1</sup>] [106].

When compared with the removal efficiency of our strain E. coli with reported strains, S6 proved highly U (VI) resistant and showed fast remediating properties. At the point when its CFE was typified in calcium alginate bioploymeric dabs, the sorption productivity was improved and the isotherm fitted well with Freundlich. Accordingly, the pressed bed could remediate 932 mg U (VI) m<sup>-3</sup> water every day with a sorption proficiency of 93.2%. The proposed bioremediation process could be considered as a "green-innovation" because of insignificant utilize of chemicals. Iron-oxide-impregnated nanoparticles of alginate [107] metal removal capacity of FeO-alginate for Cr (VI) was found to be 85%. Models used to calculate adsorption capacity: Langmuir, Freundlich 4. Bacillus cereus immobilized in calcium alginate highest value of Cr(VI) Uptake by modified calcium alginate was 92.5% [108].

**Uranium exploration activities in Egypt**: the exploration works resulted in the discovery of some low grade ore which related to various geological formations such as vein type (G. Elmissikat, metasomatizedganite (G. Um Ara)), shear zones in calc- alkaline granite and intermountain basin (G. gatta) and surficial type U deposite in sedimentary rocks (Abo zeneima, Sinai) After 1996, Nuclear Materials Authority exploration activities for Uranium resources were concentrating on the south eastern forsake of Egypt these work brought about the disclosure of Three U-Assets from south to north incorporate vein-type (G El-sella and Abu Rusheid area) and metamorphosed sandstone-type (Sikait are)

Method of bioleaching: In microbial leaching (bioleaching), metals can be removed from

expansive amounts of poor-quality minerals. Despite the fact that recuperation of metals (e.g., copper) from the wastewater of mines has been known for quite a long time, the contribution of organisms in this procedure was perceived about 40 years ago. The bacteria, which are naturally related with the stones, can prompt bioleaching by one of the accompanying ways.

Coordinate activity of microbial biomass on the ore to metal bioleaching.

Bacteria produce certain substances such as sulfuric acid and ferric iron which extract the metal (indirect action) Heterotrophic Bacteria. In practice, both the methods may work together for efficient recovery of metals.

Organisms for bioleaching: The most ordinarily utilized microorganisms for bioleaching are Thiobacillusferrooxidans and Thiobacillusthiooxidans. Thiobacillusferrooxidans is a pole molded, motile, non-spore framing, and Gram-negative bacterium. It determines vitality for growth from the oxidation of iron or sulfur. This bacterium is capable of oxidizing ferrous iron  $(Fe^{2+})$  to ferric form  $(Fe^{3+})$  and converting sulfur (soluble or insoluble sulfides, thiosulfate, elemental sulfur) to sulfate (SO<sup>2-</sup>4). Thiobacillusthiooxidans is comparable with Thiobacillusferrooxidams and grows mostly on sulfur compounds. Several studies indicate that the two bacteria T. ferrooxidans and T. thiooxidans at the point when assembled work synergistically and enhance the extraction of metals from the minerals. Other than the over two microbes, there are different microorganisms associated with the procedure of bioleaching. A chose few of them are quickly depicted beneath. Maximum mobilization of inexhaustible metals like Cu from PCB using A. acidophilum provides evidence for the efficacy of the acidophilic alphaproteo bacteria in the recovery of metals from e-waste. However, the rate of metal recovery as well as the leaching time needs to be improvised by systematic investigations employing bacterial consortium and optimizing various biotic and abiotic factors were regulating the bioleaching process [109].

Pseudomonas aeruginosa can be employed in mining low grade uranium (0.02%) ore. This organism living being has been appeared to amass around 100 mg uranium for each one liter arrangement in under ten seconds. Another living being, Rhizopusarrhizus is too successfulfor removing uranium from polluted water. Sumin et al. reported that the results for the determination of the effect of pH on Cu and Cd removal are shown at below pH-2 conditions, Cu and Cd removal efficiencies were less than 61% and 38%, respectively, while at higher than pH-3 conditions, expulsion efficiencies dramatically increased to over 95%.

These results suggest that H+ can compete with  $Cu^{2+}$  or  $Cd^{2+}$  on the surface of Ca-alginate beads and hinder  $Cu^{2+}$  and  $Cd^{2+}$  bio-sorption at low pH however,  $Cu^{2+}$  or  $Cd^{2+}$  becomes relatively exchangeable with  $Ca^{2+}$  in Ca-alginate beads at higher than pH 3 conditions [110]. From results of the batch experiments, it was found that the lowest pH limit for the use of Ca-alginate beads was pH 3. So our study on Phosphoric acid Which explain increase PH in phosphoric acid from 0.3 to 0.5 due exchanges These results suggest that H<sup>+</sup> can compete with  $Cu^{2+}$  or  $Cd^{2+}$  on the surface of Ca-alginate beads and hinder U bio-sorption at low pH U becomes relatively exchangeable with  $Ca^{2+}$  in Ca-alginate spheriouls at higher than pH 3 or lower where Even the initial pH of phosphoric crowed was 0.69 (<3 of pH), the buffer action of the Ca- alginate beads will increase the pH. Because of human exercises, gigantic amounts of poisonous metals are discharged into nature every year.

At times, these discharges are thin and very much directed, as mechanical emanations, while in different cases they are incidental and incorporate synthetic spills or despicable land transfer [111]. Heavy metals like lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, gold, silver, copper, and nickel. These toxins are gotten from mining, metallurgical, electronic, chrome tanning, materials, electroplating, and compost make like phosphate manure. Expanded because of fast industrialization and mechanical advancement, posturing noteworthy dangers to biological communities and general wellbeing in light of their poisonous quality Amounts of overwhelming metals discharged into the earth have, gathering in evolved ways of life, and tirelessness in nature [112].

Controlling overwhelming metal releases and expelling lethal substantial metals from water bodies has turned into a test for the twenty-first century, where the fate of toxic metal species after they enter the ecosystem becomes difficult. Furthermore, they spread damage as they move from one ecological trophic layer to another. Strategies utilized for substantial metal expulsion from mechanical effluents can be grouped as physical, chemical, and biological origin. Physicochemical methods such as precipitation, particle, filtration, membrane and electrochemical technologies, reverse osmosis, electro- dialysis, adsorption on activated carbon, etc. have disadvantage, which is high capital and working expenses and may be related with the generation of secondary wastes, which cause treatment problems. Therefore, recent attention has been drawn towards the improvement of option methodologies known as bioremediation processes.

A solubilizing process was done utilizing glucose oxidase protein immobilized in calcium alginate beads shows bioleaching performance of immobilized glucose oxidase enzyme. Almost similar metal recovery was achieved using free and immobilized glucose oxidase catalyst. A slight decrease in metal recuperation was observed in the second bioleaching cycle. The metal recovery decreased drastically in 3rd and 4th bioleaching cycle. Further poor metal recovery was observed in fifth bioleaching cycle. Low stability and high porosity of the membrane limit use of encapsulation process for immobilization of enzymes.

#### Plan of the Work

Screening of uranium-resistant bacterial isolates growth in presence of different uranium optimum condition for bacterial isolates optimum condition for bio-sorption. Process analyzed the FTIR spectra and energy dispersive X-ray (EDX) of U loaded and unloaded of free and immobilized beads like capsule of the bacterial isolates Desorption ratio of uranium ions adsorbed by E. coli or alginate microcapsule beads recycle Isolation and biochemical, molecular identification of novel bacterial isolate which isolated from rock sample.

Total protein estimation in filtrates of free and immobilized bacteria incubated with U as single metal or mixed environmental applications using immobilized bacteria isolated from the uranium ore for bio- sorption of uranium. Factors affecting uranium bio-sorption, biomass concentration, time, pH and temperature, while other factors belong to types of biomass such as living or dead, free or immobilized cells, and the bio-sorptive capacity of cell biomass. Optimization condition for uranium bioleaching efficiency by immobilized bacterial isolates. Various isotherm kinetic equations of adsorption such as, Langmuir, Freundlich relation between bioleaching efficiency and bacterial by- products: There are several benefits of using enzymes for environmental applications.

#### **CONCLUSION:**

Bacteria a Microscopic organisms are the most inexhaustible and flexible of microorganisms and constitute a huge division of the whole living earthly biomass, certain microorganisms were found to amass metallic components at a high limit Was Known as Bacterial Bio-sorption Due to their little size, capacity to become under controlled conditions, and their Accommodation to an extensive variety of ecological situations; Potent metal bio-sorbents 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 increment bio-sorption onto the cell surface. Starting with Isolation and identification of heavy metal-resistant bacteria from rock Ore. Studying Factors Affecting Uranium Bio-sorption, Optimization of bacterial growth conditions and optimum for metal uptake by free and immobilized bacterial cells and Desorption ratio of uranium ions adsorbed by Coli. /alginate, 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 it's groups confirm bio-sorption 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 bio-sorption of metal ions from waste due to ions charge interaction.

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