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RESEARCH ARTICLE

BIOSORPTION OF COPPER AND LEAD USING BACTERIAL BIOMASS OF *BACILLUS CEREUS* AND *BACILLUS SUBTILIS* ISOLATED FROM EL-MANZALA LAKE, EGYPT.

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Abstract

Two tolerant bacterial strains isolated from El-Manzala Lake, Egypt and identified using biochemical tests and confirmed by 16S r RNA gen**e** as *Bacillus cereus* and *Bacillus subtilis*.Then the optimum conditions for biosorption of copper and lead were investigated by using two bacterial strain, the equilibrium time for copper were 25 minutes at *Bacillus cereus*and 30 minutes at *Bacillus subtilis* while the equilibrium time for lead were 40 minutes at *Bacillus cereus*and 50 minutes at *Bacillus subtilis*, the optimum pH for copper and lead biosorption at *Bacillus cereus* and *Bacillus subtilis* was pH 6. The experimental biosorption data mostly were fitted towards the models postulated by Langmuir and Freundlich isotherm equations. The maximum biosorption capacities (q_{max}) for copper and lead obtained by using*Bacillus cereus*were 47.6 and 250 mg/g while by using*Bacillus subtilis*were 166.7 and 250 mg/g , respectively. Biosorpitve mechanism was confirmed by IR analysis and from the identification nature of acidic and basic sites. Moreover, the postulated mechanism was depended mainly on ionic interaction and complex formation. The results demonstrated that the two bacterial isolates of Bacillus cereus and Bacillus subtilis could be used as a promising biosorbents for the removal of copper and lead ions from aqueous solutions.

Introduction:-

Industrial operations such as electroplating, steel manufacturing, leather tanning, wood preservation, ceramics, glass manufacturing and chemical processing and fertilizer applications release alarmingly higher amounts of heavy metals into the natural environment (**Zoubolis*et al.*, 2004; Khan *et al.*,2009; Oliveira *et al.*, 2011; Tian*et al.*, 2012).**

As potentially harmful and non-biodegradable pollutants that may accumulate through the food chain, heavy metals can threaten ecosystem and human health (**Liu *et al.*, 2013**).

Conventional methods for removing metals from aqueous solutions include chemical precipitation, chemical oxidation or reduction, ion exchange, filtration, electrochemical treatment, reverse osmosis, membrane technologies, and evaporation recovery. These processes may be ineffective or extremely expensive; especially when the metals in solution are in the range of 1–100 mg/l, the production of toxic chemical sludge and its disposal/treatment becomes a costly affair and is not ecofriendly (**Nourbakhsh*et al.*, 1994**).

Therefore, removal of toxic heavy metals to an environmentally safe level in a cost effective and environment friendly manner was of great importance. Biological treatment, based on living or nonliving microorganisms or

plants, offers the reduction of toxic metal levels to environmentally acceptable limits in a cost-effective and environmentally friendly manner (**Volesky, 1994**).

]Biosorption has emerged as an alternative solution for the removal of toxic metals from water/wastewater. It shows superiorities in low cost, high efficiency, wide adaptability, no secondary pollution, and stable performance especially for low metal concentration effluents (**Wang and Chen, 2009**).

Nowadays, the use of microbial approaches for heavy metal removal has received much attention .Bacteria, algae, fungi, and yeasts constitute a wide range of biosorbents with different adsorption capacities. These capacities depend on the cell wall structure and the affinity of surface ligands to specific metal ions. Different parameters such as tendency toward the metal ions, the maximum sorption capacity, as well as the rate of the metal sorption on the surface of the biosorbents are the major criteria for comparing and choosing the best type of biosorbents for specific purposes. Using the equilibrium isotherms and kinetic studies are common for the calculation of these parameters. (**Joo*et al*., 2010).**

The bacteria make excellent biosorbents because of their high surface-to-volume ratios and a high content of potentially active chemo sorption sites such as on teichoic acid in their cell walls (**Beveridge, 1989**).

The main objective of this work is to study biosorption processes of copper Cu (II) and lead Pb (II) used Bacillus cereus and Bacillus subtilis. The conditions of these processes were optimized by selection of both the pH and time postulated at room temperature. Also study the biosorption isotherm and the mechanism of the process was discussed.

Materials and Methods:-

Isolation of heavy metal resistant bacteria:-

Heavy metal resistant bacteria were isolated from heavy metal polluted water samples collected from El-Manzala Lake, Egypt , In order to minimize the complexation of heavy metals, the isolates were grown in Tris minimal medium (Tris-HCl-100 (pH-7.2), Glucose-11, NH4Cl2-4 , MgCl2-10, CaCl2-0.1, KH2PO4-0.1, in millimoles per liter of deionized water and Agar-15g/L) (**Mergeay, 1995**). For isolation of bacteria by agar dilution method, plates were inoculated with 200 µl from sample by spread technique on Tris minimal medium supplemented with different concentration of heavy metals from Cu (II) and Pb (II), one metal at a time. Plates were incubated for 72 hrs at 37°C.After the incubation, the plates were examined and the bacterial isolates were picked and transferred to agar slants and then purified by streaking several times on nutrient agar media, until pure single colonies were obtained. Isolates were maintained in slope culture and stored at 4 ^{o}C for further studies.

Preparation of metal solutions:-

1. A stock solution of copper Cu (II) 1000 mg / l was prepared by dissolving 3.929 g $CuSo_4 .5H_2O$ (M.W = 249.68) in 1000 ml of deionized water .
2. A stock solution of lead Pb (II) 1000 mg / l was prepared by dissolving 1.598 g $Pb(No_3)_2$ (M.W = 331.21) in 1000 ml of deionized water .

The chemicals used for this study were of analytical grade and they were supplied by Sigma Aldrich (**Sigma Aldrich, St.louis, Mo**) .The heavy metals were sterilized by filtration method. The solution metals pass through a membrane filter (pore size 0.45 µg).

Identification of the metal resistant bacterial isolates:-

The most resistant bacterial isolates were identified according to the keys of Bergey's Manual of Systematic Bacteriology (**Holt *et al*., 1986**)and according to Bergey's manual (2010), These tests including: Gram staining, spore staining, motility and biochemical testes; indol, VogesProskaur, nitrate reduction ,citrate utilization, urease, gelatinase, oxidative fermentation ,hydrolysis of casine and hydrolysis of starch,……. etc. bacterial isolates also were identified by usinga matrix ofAPI 20E strip and the API 50 CHB strips (**bioMérieux, France**).

Confirmation of identification using Molecular Identification by 16S r RNA gene sequencing:-

16S rRNA gene sequence analysis is a widely accepted tool for molecular identification (**Bo¨ttger 1996, Kolbert*et, al* 1999, Patel 2001**).Public databases (GenBank, Nucleotide Sequence Database at the European Molecular Biology Laboratory, DNA Data Bank of Japan, RDP II) contain a vast number of bacterial 16S rRNA sequences, allowing

for rapid analysis and providing phylogenetically meaningful information. To determine the 16S rRNA gene sequence of the strain, cells were lysed according to **Hiraishi*et al*.** The 16S rDNA fragment was amplified by PCR using the following universal primers: forward, 59-AGAGTTT–GATCATG GCTCGA -39 ; and reverse, 59-GGCTACC-TTGTTACGACTT-39 (positions 1510 - 1492). The sequence of the amplified 16S rDNA fragment was analyzed using gene bank and compared with the National Center for Biotechnology Information (NCBI) database.

Biosorbents preparation:-
Nutrient medium was prepared and sterilized. A loop full of bacterial culture was taken and streaked on the agar plate to obtain more colonies. They are later transferred to nutrient broth for subculture. 100 ml of sterilized culture media was transferred to 250 ml Erlenmeyer flask. The media was allowed to cool and then the 100µ microbial solution was inoculated into the medium in laminar air flow chamber.

The inoculated flasks were incubated in an orbital shaker at 250 rpm at 32^0C for 2 days to obtain the biomass. Biomass was harvested from the medium by centrifugation at 9000 rpm for 10 min. The supernatant was discarded and the cells pellet was rinsed three times with sterilized water to make sure that no media remain on the cell surface. Then using a lyophilizer to freeze and dry bacterial biomass which was used for the sorption experiments.(**Aksu and Donmez 2001**).

Effect of contact time on biosorption.
The freezed dried cells (50mg) were inoculated to a series of Erlenmeyer flasks (9 flask for each metal) containing the diluted solution (50ml) with 50 ppm of each heavy metal were studied (Cu II and Pb II) then the flask were shaken (150 rpm/min) at 30 °c for a certain time (0 – 60min) .The cell solution (20 ml from each flask)at the time intervals (0, 5, 10, 15, 20, 25, 30, 40, 50 and 60 min) was filtered through 0.2 µm filter membranes and the supernatant was analyzed for measure remaining metal ions concentration using Atomic absorption spectrophotometer (**Perkin Elmer A Analyst 200**).Blank experiments were conducted to ensure that is no adsorption had taken placed on the walls of apparatus used (**sang *et al*., 2009**).

Effect of pH on biosorption:-
The metals sorption monitored for pH range 2 to 6 for each metal were studied, and 50 mg from lyophilized cells were inoculated in the metal solution at initial concentration 200 mg /L for each metal at 30 ° C at the equilibrium time. After filtration through 0.2 µm filter membranes and the supernatant was analyzed for measure remaining metal ions concentration **(Sang *et al*., 2009**).

Effect of initial metal concentration on biosorption:-
To study the effect of different concentrations of metals 50 mg from biomass were inoculated in the metal solution at different concentration (50,100,150,200,250 and 300) mg /L for each metal at 30 °C at the equilibrium time .The solutions for each metal were adjusted to the optimum pH in which maximum biosorption of the metal ion occurred. After filtration through 0.2 µm filter membranes and the supernatant was analyzed for measure remaining metal ions concentration. (**Sang *et al*., 2009**).

Data evaluation:-
The effect of contact time, initial concentration and pH on metal adsorption was calculated using the following equation:

$$\text{Metal adsorbed (\%)} = \left(\frac{C_{i-C_e}}{C_i}\right) \times 100$$

Amount of metal adsorbed by bacterial biomass was calculated from the differences between the metal quantity added to the biomass and the metal content of the supernatant.

The specific metal biosorption q was calculated using the following equation:

$$q_e \text{ (mg / g)} = \left\{\frac{C_{i-C_e}}{M}\right\} \times V$$

Where q_e is the specific metal biosorption (mg metal / g biomass), C_i and C_e are the initial and equilibrium concentrations of metal (mg metal /l) respectively, V the volume of metal solution (l) and the M is the dry weight of biomass (g) in grams.

Biosorption isotherm:-

The biosorption equilibrium isotherm was obtained by Freundlich model (equation 1) and the Langmuir model (equation 2) respectively (**Volesky1990**).

Mathematical formula for Freundlich model can be expressed as:

$$q = K_f C_e^{\frac{1}{n}} \qquad \textbf{(1)}$$

Where K_f and n are the distribution coefficient and a correction factor, respectively .By plotting the linear form of Eq. (1) $\ln q = 1/n \ln C_e + \ln K_f$ The slope is the value of $1/n$ and the intercept is equal to$\ln K_f$.

And mathematical formula for Langmuir model can be expressed as

$$\mathbf{q_e = \frac{q_{max} b C_e}{1 + b C_e}} \qquad \textbf{(2)}$$

And it's linear form is represented by the following equation**:**

$$\mathbf{\frac{C_{eq}}{q_{eq}} = \frac{1}{q_{max} b} + \frac{C_{eq}}{q_{max}}} \textbf{(3)}$$

Where q_{max} is the Langmuir constant (mg/g) reflecting the maximum adsorption capacity of the metal ion per unit weight of biomass to form a complete monolayer on the surface bound at high C_{eq} . The value of Langmuir constantb (l/mg) represents a ratio of adsorption rate constant to desorption rate constant, which also gives an indication of the affinity of the metal for binding sites on the biosorbents.q_{max} andb can be determined from the linear form of Langmuir equation (3) by plotting$\mathbf{\frac{C_{eq}}{q_{eq}}}$**vs.** $\mathbf{C_{eq}}$.

FT-IR analysis:-

Samples were analyzed using Fourier transform Infrared (FT-IR) spectroscopy to give a qualitative and preliminary characterization of the main functional chemical groups present on the bacterial biomass, which are responsible for heavy metal biosorption. A raw sample of bacterial biomass and biomass loaded with different heavy metals were analyzed using FT-IR (**Perkin Elmer, FT-IR system, Spectrum BX)** adopting KBr disk technique.

Results and discussion:-

Characteristics of biosorbents:-

Bacillus cereus and Bacillus subtilis used in this study were previously isolated from polluted water samples collected from El-Manzala Lake, Egypt. Then two bacterial strains were characterized by microscopic examination and biochemical tests as well as identified by a matrix of API 20E strip and the API 50 CHB strip (**bioMérieux, France**)**.**

Identifications were confirmed by16S r RNA gene sequencing **,**nucleotide sequence coding for 16SrDNA gene has been submitted to GenBank and the strains is closely related to *Bacillus cereus* and *Bacillus subtilis* (with similarities of 98% and 98% respectively) as shown in figures (**1,2**) phylogenetic tree based on 16S rRNA gene sequences .

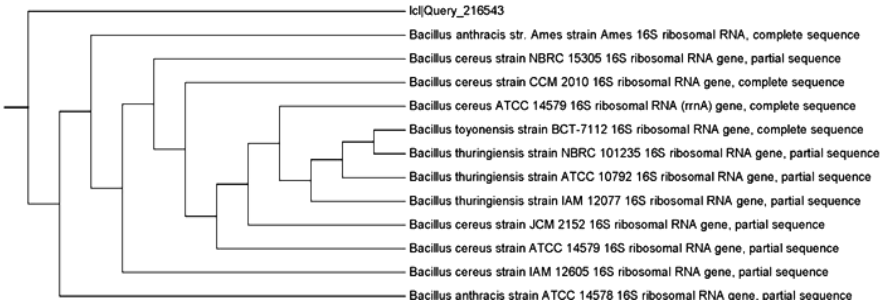


Figure 1: Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationships of *Bacillus cereus*and other related taxa.

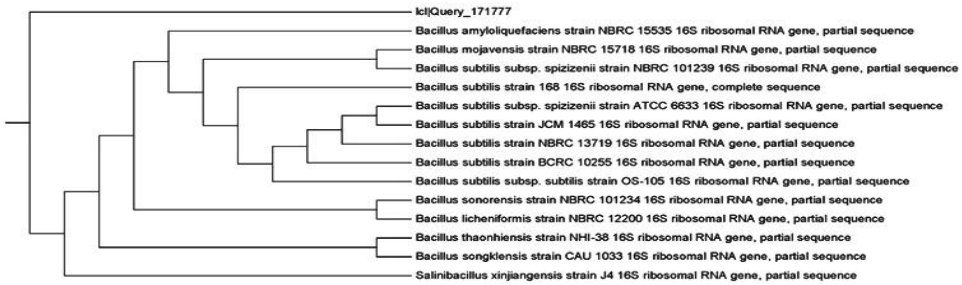


Figure 2: Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences, showing the relationships of *Bacillus subtilis*and other related taxa.

Biosorption experiments:-

Effect of contact time on biosorption:-

Contact time is one of the important parameters of successful biosorption application. Figures (3, 4) shows biosorption of Copper and lead by two bacterial biomass depending on time .The equilibrium time at which equilibrium metal ion concentration was presumed to be have been attained .Figures (3, 4) showed that the rate of metal uptake increased rapidly in the first part within 5 minutes of contact .after that the rate of biosorption decreased until reach a constant value of metal concentration that's called equilibrium time, the results showed that the appropriate equilibrium time for copper were 25 minutes at *Bacillus cereus* and 30 minutes at *Bacillus subtilis*. This short time required for biosorption is in accordance with the result given by other authors (**Chen *et al.*, 2005 ;O¨ztu¨rk*et al.*,2004; Pardo*et al.*, 2003;Dundar*et al.* 2007**;**Tsezos and Volesky, 1982**),while the equilibrium time for lead were 40 and 50 minutes at*Bacillus cereus* and *Bacillus subtilis*respectively ,the order of biosorption rate was Cu> Pb These indicate the equilibrium time at which an equilibrium metal ion concentration is presumed to have been attained. In this context, (**Zouboliset *al.*, 2004; and Volesky 1990)**here also observed that the initial shortest time period of sorption process is important for a high rate of metal sorption. Similar results have also been determined by (**Gabr*et al.*, 2008)**for Pb biosorption.

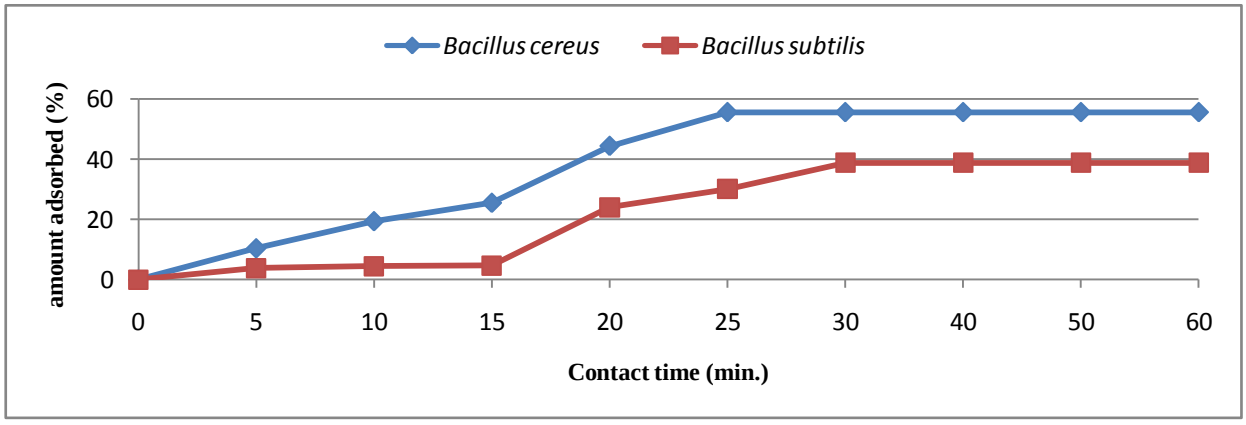


Figure 3:- Biosorption of copper (Cu II)by lyophilized cells of *B.cereus* and *B.subtilis* over the reaction time at initial concentration of metals 50 mg/l, , biomass concentration 50 mg/l and 30 ºC.

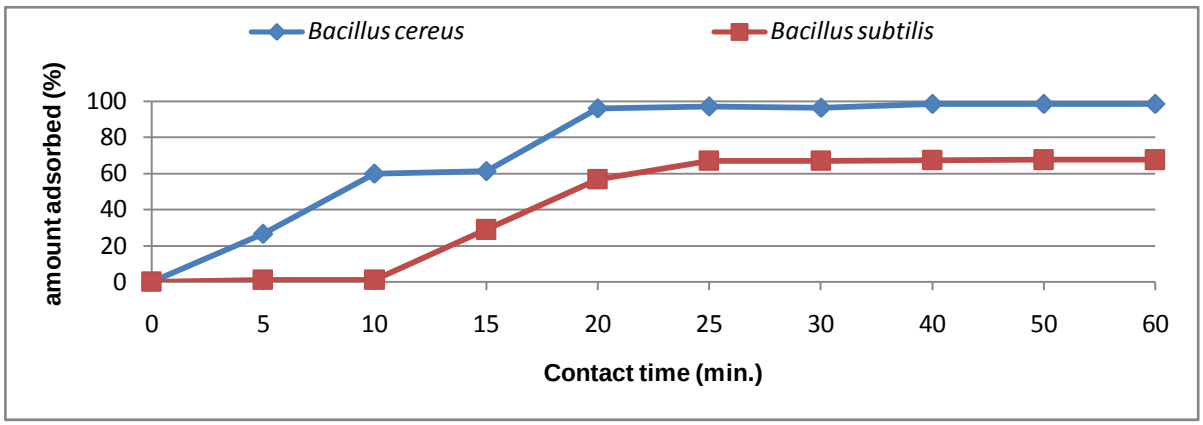


Figure 4:- Biosorption of lead (Pb II) by lyophilized cells of *B. cereus* and *B. subtilis*over the reaction time at initial concentration of metals 50 mg/l, , biomass concentration 50 mg/l and 30 ºC.

Effect of pH on biosorption:-

Figures (5,6) summarizes the results of the adsorption of Cu (II) and Pb (II) ions by*Bacillus cereus* and *Bacillus subtilis* as a function of pH. In all cases,It has been shown that the affinity of cationic species towards the functional groups present in the cellular surface is strongly dependent on the pH (**Schiewer and Volesky, 1995**).

Metal uptake by the biomass increases with increasing pH till it reaches a maximum after which the metal uptake decreases. The optimal pH values for Cu (II) and Pb (II) by *Bacillus cereus* and *Bacillus subtilis* adsorption were pH 6 , these results suggest that the adsorption of metals on the biomass surface is controlled by ionic attraction. At low pH values, the inactivated cell surface becomes more positively charged, leading to reduce the attraction between metal ions and functional groups at the cell wall. In contrast, when the pH increases, the cell surface is more negatively charged and the process of retention is favored (**Pardo*et al.*, 2003; Volesky and Holan, 1995**) until a maximum is reached around pH 6 However for values of pH higher than the optimum, the formation of hydroxylated complexes of the metal will also compete with the active sites and as a consequence, the retention will decrease again. Copper and Lead biosorption is maximized at pH 6 ,this is in agreement with the results obtained by **Pardo*et al.* (2003),** who found that the maximum pH for lead by *P. putida* is pH 6. Moreover, **Seki *et al.* (1998)** studied the function of pH on biosorption of lead by *Rhodobacter sphaeroides* and reported that the maximum pH is around 6, the variation in biosorption of heavy metals by microbial biomass at different pH could be due to the differences in the sensitivity of cell wall molecules of the bacterial cells to pH. For instance, at a low pH, cell wall ligands tightly bind with the hydronium ions H3O_ and hence restrict the approach of metal cations due to repulsive force. On the contrary, at higher pH values, more ligands like carboxyl, phosphate, imidazole and amino group would be exposed and carry negative charges with a subsequent attraction of metallic ions with positive charge and biosorption onto the cell surface (**Pardo*et al.*, 2003**).

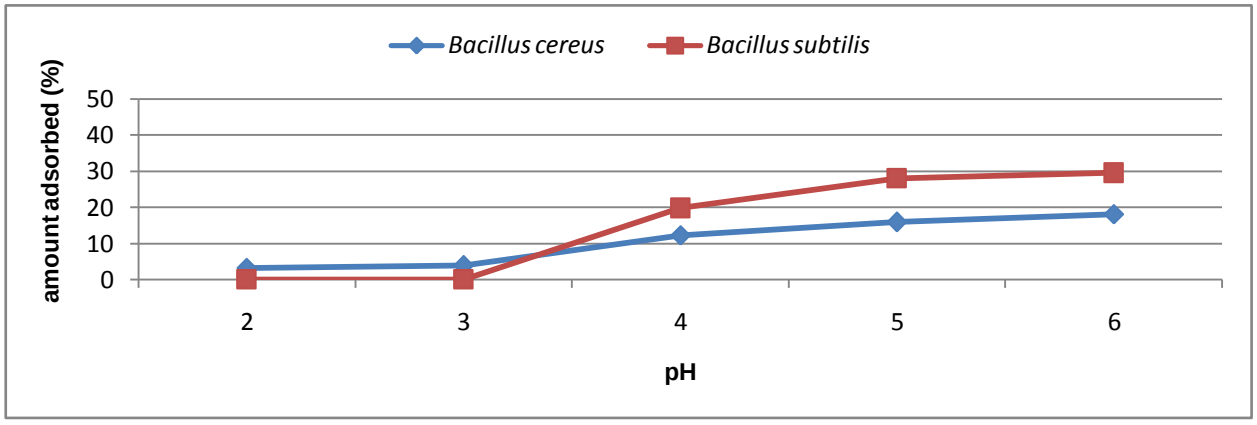


Figure 5:- Effect of pH on biosorption of copper (Cu II) by *B. cereus* and *B. subtilis*at initial concentration of metals 200mg/l , biomass concentration 50 mg/l , equilibrium time 25 min. for *B. cereus*, and 30 min. for *B. subtilis*and 30° C .

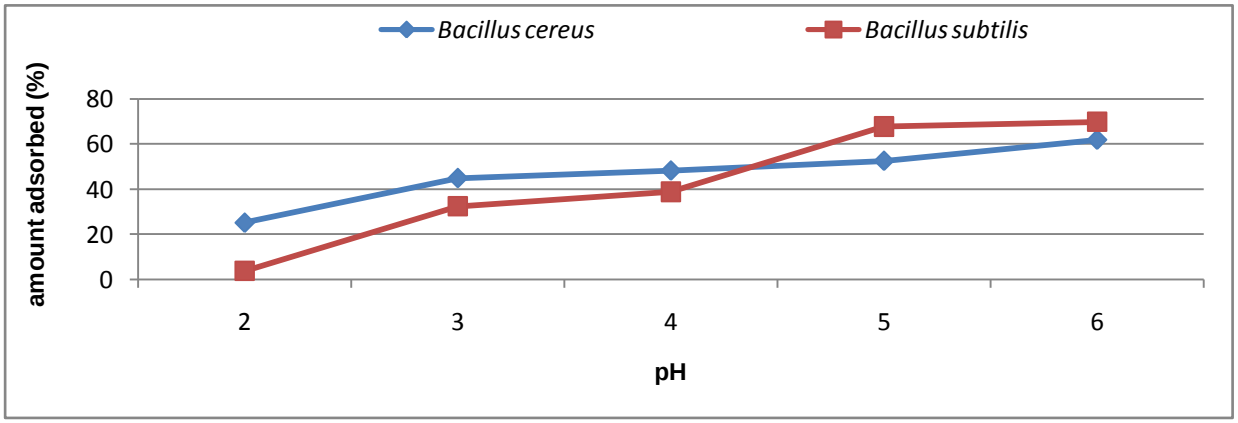


Figure 6 : Effect of pH on biosorption of lead (Pb II) by *B.cereus* and *B. subtilis*at initial concentration of metals 200mg/l , biomass concentration 50 mg/l , equilibrium time 40 min. for *B. cereus* and 50 min. for *B.subtilis* and 30° C **.**

Effect of initial metal concentration on biosorption:-

The effect of initial metal concentration on metal biosorption by dry biomass of *B.cereus* and *B.subtilis*were evaluated as shown in Figures (7,8)that's indicate the rate of biosorption decreased with an increase in metal ion concentration. The maximum biosorption percentage of metal was recovered at a low initial metal ion concentration; the decrease in the percentage of biosorption may be attributed to the lack of sufficient free sites for metal biosorption. At lower concentrations, all metal ions present in the solution however, could interact with the binding sites and thus the biosorption percentage is likely to become higher than that at higher ion concentrations as found in this study .At higher concentrations, a lower adsorption yield is due to the saturation of adsorption sites. Similar results have been reported by others (**Kadukova and Vircikova, 2005; Lu *et al.*, 2006; Pandiyan and Mahendradas, 2011**).

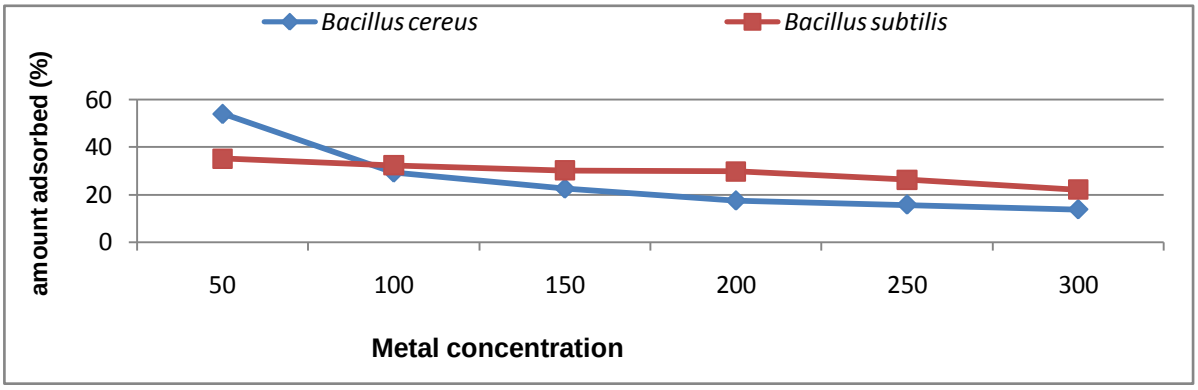


Figure 7:- Effect of initial concentration of copper (Cu II) on biosorption by lyophilized cells of *B. cereus* and *B. subtilis*.at 50 mg biomass, 30 ^{o}C temperature, pH 6 , 25 min .for*B.cereus* and and 30 min. for*B. subtilis*.

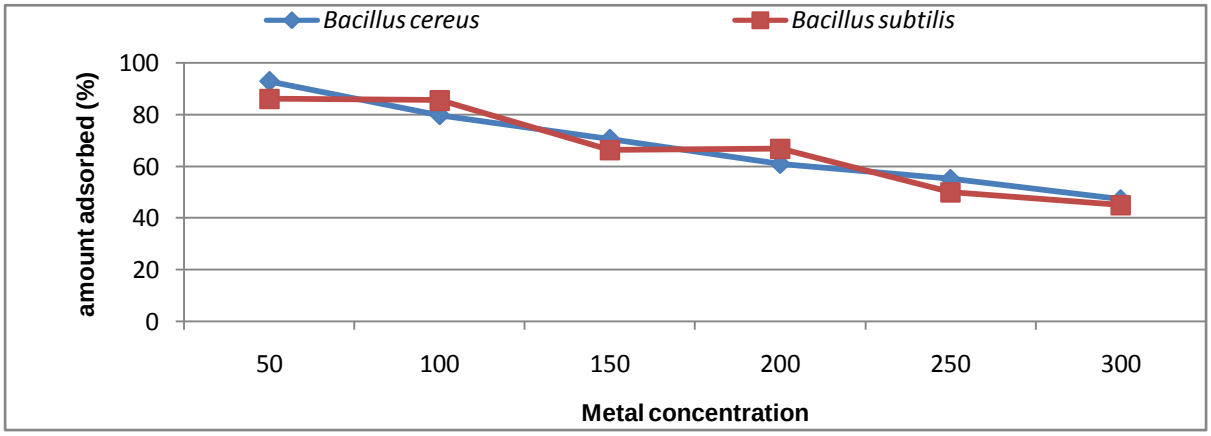


Figure 8: Effect of initial concentration of lead (Pb II) on biosorption by lyophilized cells of *B. cereus*and *B. subtilis* and at 50 mg biomass, pH 6, equilibrium time 40 min. for *B. cereus*, 50 min. for *B. subtilis* and 30^{o} C temperature.

Biosorption isotherm:-

The biosorption isotherm models described the biosorption data at equilibrium and showed the correlation between the mass of solute adsorbed per unit mass of sorbent at equilibrium .The biosorption isotherms were calculated using two different isotherms models including the Langmuir, and Freundlich, Figures (9, 10, 11 and 12)The equilibrium adsorption isotherm obtained showed that metal uptake by bacterial biomass was a chemically equilibrated and saturabled mechanism. Thus, there was an increase in metal uptake as long as binding sites were free. Values of Freundlich and Langmuir parameters are calculated and listed in Table (1), These data showed that the q_{max} values obtained for lead uptake using lyophilized biomass of *Bacillus cereus* and *Bacillus subtilis* were 250 mg metal/g biomass, which were higher than those obtained for copper : 47.6 ,166.7 mg metal/g biomass respectively. However, the b values obtained were found to be 0.003, 0.004respectively, in the case of lead biosorption, where copper biosorption b values were recorded 0.018, 0.002respectively, which indicate that *Bacillus cereus* possesses a high adsorption affinity for copper as compared to that for lead, in contrast of *Bacillus subtilis* which possesses a high adsorption affinity for lead as compared to that for copper .The values of Freundlich parameters show that the adsorption capacity K_f for copper using*Bacillus cereus* and *Bacillus subtilis* were 10.01 and 0.88 mg metal/g biomass, respectively, where The values obtained for lead, 3.59, 2.83, mg metal/g biomass respectively, However, the small K_f values for copper ions at*Bacillus subtilis*indicate a lower extent sorption, while more sorption was observed for copper ions at*Bacillus cereus* because of their larger K_f values .that's also observed in lead adsorption by the two bacterial strains .Here it is worth mentioning that the correlation coefficients for all the copper and lead by *Bacillus cereus* were found to be 0.948 and 0.986 respectively ,while the correlation coefficients for copper and

lead by *Bacillus subtilis* were found to be 0.980 and 0.922 respectively , In general, these data indicated that the sorption capacity increased with increasing the initial metal ion concentration for both metals on the biomass surface for the two organisms . This sorption characteristic indicates that the surface saturation is dependent on the initial metal-ion concentrations. At low concentrations, adsorption sites took up the available metal more quickly. However, at higher concentrations, metals need to diffuse into the biomass surface by intra particular diffusion and greatly hydrolyzed ions will diffuse at a slower rate (**Gaber*et al.*, 2008**, **Sang *et al.*, 2009).**The overall observation from bothLangmuir and Freundlich isotherms for metals biosorption were in agreement with many previous studies (**Pardo *et al.*, 2003, M. Acosta *et al.*, 2005,Sar*et al.*, 1999).**

This preferential type of adsorption may be ascribed to the difference in their ionic radii(**Gaber*et al.*, 2008**).The ionic radius of Cu (II) is 0.37Å,while that of Pb (II) is 1.20 Å.

Mohammad*et al.*, (2012) reported thatthe biomass of *Bacillus thuringiensis* OSM29 successfully removed the metals such as Cd, Cr, Cu, Pb and Ni from aqueous solution.
Su, *et al.*, (2014) used*Bacillus catenulatus* for biosorption of cadmium and the biosorption equilibrium data were well fitted by the Langmuir adsorption isotherm,

Table 1: Freundlich and Langmuir isotherm parameters for Copper and lead biosorption by bacterial isolates.

Metal	Organism	Freundlich			Langmuir		
		K_f	n	r^2	b	q_{max}	r^2
Copper	*Bacillus cereus*	10.01416	4.115226	0.948	0.018551	47.6	0.982
	Bacillus subtilis	0.876341	1.283697	0.980	0.002467	166.7	0.892
Lead	*Bacillus cereus*	3.589454	1.529052	0.986	0.00325	250	0.99
	Bacillus subtilis	2.832048	1.436782	0.922	0.00492	250	0.917

$K_f$sorptive capacity , n sorptive intensity , r^2 correlation coefficient , b Langmiur constant , q_{max} maximum adsorption capacity .

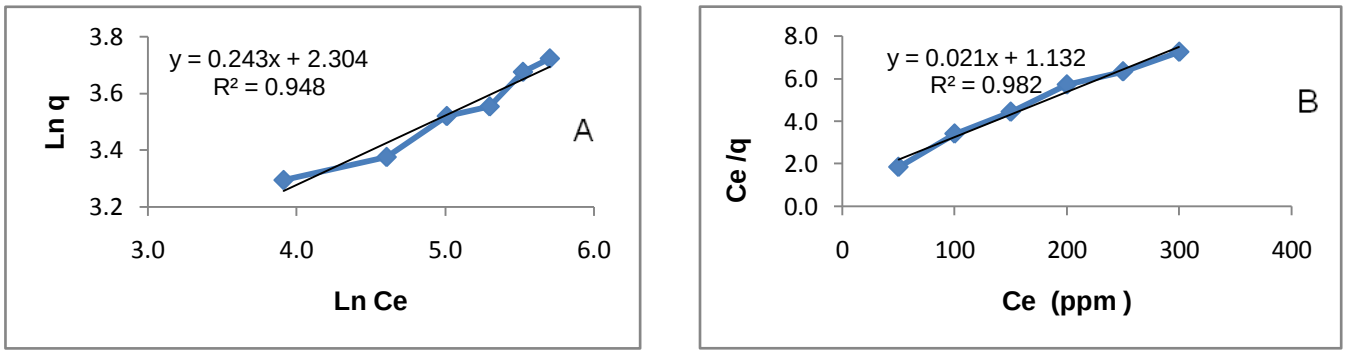


Figure 9: Adsorption isotherm (A) linear form of Freundlich, (B) linear form of Langmuir for Copper (Cu^{2+}) by *Bacillus cereus*at 50 mg biomass, 30 ^oC temperature, pH 6 , 25 min.

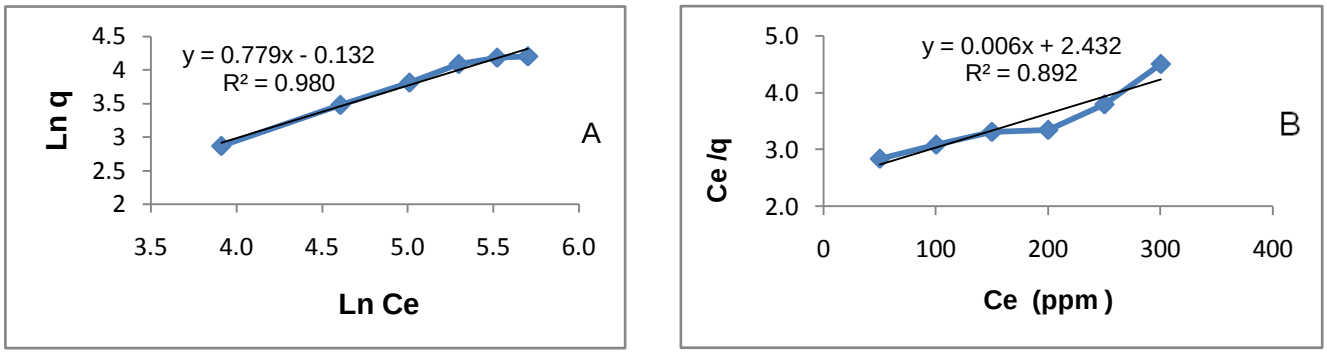


Figure 10: Adsorption isotherm (A) linear form of Freundlich, (B) linear form of Langmuir for Copper (Cu^{2+}) by *Bacillus subtilis* at 50 mg biomass, 30 ^oC temperature, pH 6 , 30 min.

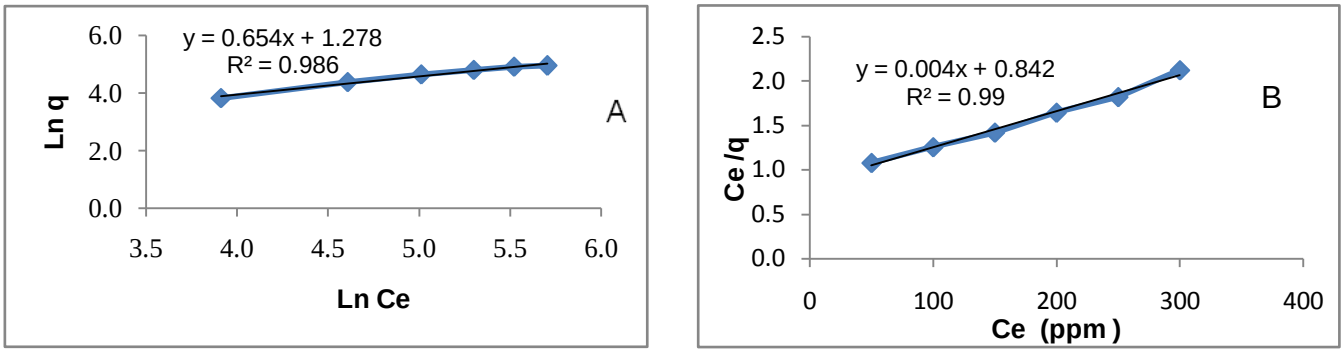


Figure 11: Adsorption isotherm (A) linear form of Freundlich, (B) linear form of Langmuir for Lead (Pb^{2+}) by *Bacillus cereus*at 50 mg biomass, 30 ^{o}C temperature, pH 6 , 40 min.

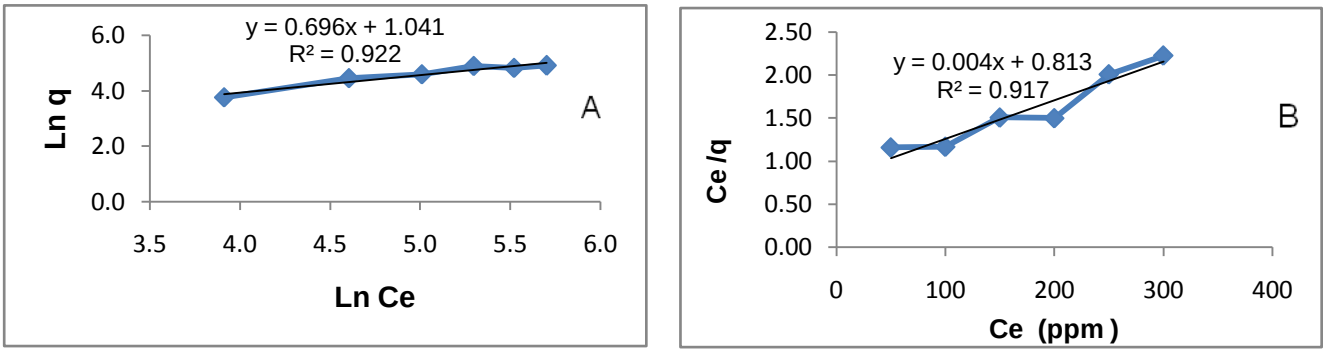
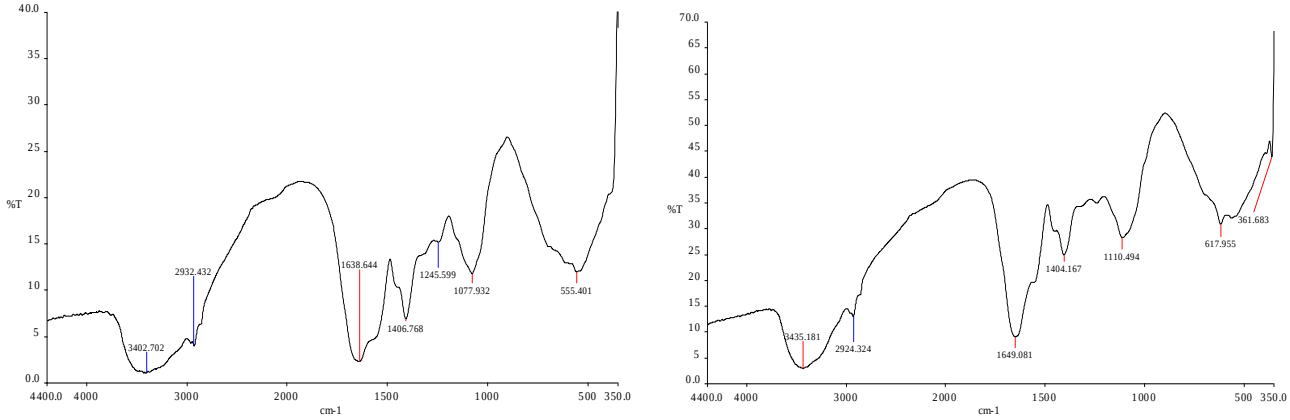


Figure 12: Adsorption isotherm (A) linear form of Freundlich, (B) linear form of Langmuir for Lead (Pb^{2+}) by *Bacillus subtilis*at 50 mg biomass, 30 ^{o}C temperature, pH 6 ,50 min.

FTIR Analysis:-

It is essential to identify the functional groups on the biomass involved in adsorbing process with FTIR, which is helpful to understand the surface-binding mechanism. FTIR spectrum in the absence and presence of metal revealed the changes in the peaks of functional groups at bacterial biomass, for example FTIR spectrum for *Bacillus cereus* biomass before and after adsorption of copper were occurred , Figure (13) showed FTIR spectrum in the absence and presence of Cu (II) which revealed the changes in the peaks of functional groups , such as a shift from 3402 cm^{-1} to 3435 cm^{-1} indicating hydroxyl O–H stretch, H–bonded , also the absorption bands characterizing alkyl chains and CHO have abroad band within the range 2932-2924 cm^{-1} , c=o of amide group at 1638 -1649 cm^{-1} ,COO^{-} of the carboxylate groups appeared at 1406- 1404 cm^{-1} , That vibrations from1077 cm^{-1} (before Cu (II) adsorption) to 1110 cm^{-1} (after Cu (II) adsorption) could be caused by C–N stretch, The peak at 555 cm cm^{-1} shifted to 617 cm^{-} after Cu (II) adsorption could be assigned to the stretching of C–O (carboxyl).The overall FTIR spectra analysis implied that the functional groups like hydroxyl, carbonyl and carboxyl may be involved in Cu (II) adsorption. Therefore, infrared spectra of *B. cereus*biomass showed the presence of amine R–NH2 (amino acids, proteins, glycoproteins, etc.), carboxylic acid (fatty acids, lipopolysaccharides, etc.), hydroxyls, and phosphates .In general the transmittance of the peaks in the loaded biomass is substantially lower than those in the raw sample of the bacterial biomass , this indicated that bond stretching occurs to a lesser degree due to the presence of metals and following peak transmittance is reduced ,these result s are in agreement with **Norton *et al.*, (2004) and Sang *et al.*, (2009).**The above observations indicated the involvement of these functional groups in the biosorption process .These results are in good agreement with those obtained by other authors (**Gaber*et al.*, 2008,Komy *et al.*, 2006,Lodeiro *et al.*, 2006 ,Tunali*et ,al* .2006)** who concluded that the main functional groups responsible for biosorption of heavy metals are carboxylic ,hydroxyl and amino groups.Also**Mohammad*et al.*, (2012)** reported thatthe functional groups identified on bacterial surface by FTIR technique(for *Bacillus thuringiensis*) included amino, carboxyl, hydroxyl and carbonyl groups, which could possibly be involved in the biosorption of Cd, Cr, Cu, Pb and Ni.



AB

Figure 13: FT-IR analysis for *Bacillus cereus* biomass (A) (metal free) and biomass loaded with 100 ppm of Copper (B).

Conclusions:-

The present work was designed to investigate the biosorption behavior of Cu (II) and Pb (II) to the gram positive bacteria *Bacillus cereus* and *Bacillus subtilis*. The optimum pH for copper biosorption is 6 , at temperature 30° C ,equilibrium time 25 minutes for *B. cereus*, and 30 minutes for *B. subtilis*, while the optimum pH at *Bacillus cereus* and *Bacillus subtilis* for lead biosorption is 6 , at temperature 30° C ,equilibrium time 40 minutes for*B. cereus*, and 50 minutes for *B. subtilis*. The maximum biosorption capacities for *Bacillus cereus* and *Bacillus subtilis* were 250 mg metal/g biomass for Pb at optimum operating conditions, while for Cu were 47.6, 166.7 mg metal/g biomass respectively. The experimental data revealed that Cu and Pb biosorption mostly were fitted to both Freundlich and Langmuir isotherms.

The mechanism of biosorption includes mainly ionic Interactions and formation of complexes between metal cations and acidic sites in the cell wall of bacterium, and this was confirmed by IR and pH experiments.IR spectroscopy result shows that the rod-shaped *B.cereus*cell mainly contains carboxyl, hydroxyl, phosphate, amino, and amide functional groups. Based on these results *Bacillus cereus* and *Bacillus subtilis* biomass can be used as an efficient low cost biomass for the removal of heavy metals from wastewater. Finally the results demonstrate that bacterial isolates of *Bacillus cereus* and *Bacillus subtilis*could be used as a promising biosorbents for the removal of copper and lead ions from aqueous solutions.

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