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RESEARCH ARTICLE

REMEDIATION OF CADMIUM IONS FROM THE CONTAMINATED AQUEOUS MEDIA BY USING DEAD BIOMASS OF*BACILLUS SUBTILIS*

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Abstract

Aquatic systems are severely polluted by heavy metal pollutants which results in worsening the quality of water which arises as a serious threat that needs to cope up by the world. These toxicants show detrimental effects on all life forms which leads to disturbance of the ecosystems. Therefore, there is a vital need to clean the polluted environment by developing new innovative approaches for the remediation of water systems. In the current study,batch experiments were performed to check the potential of biosorbent*Bacillus subtilis*for the biosorption of cadmium metal ions from contaminated aqueousmedia and the favorable environment for the highest removal (87.50 %) of cadmium ions were obtained after 60 minutes of equilibrium time at pH 5, 0.2 g biomass and 20 mg/L of cadmium concentration. The experimental adsorption data were better fitted towards the Langmuir model for Cd (R^2 = 0.9938)than the Freundlich model, while the data related to chemical kinetics obtained by pseudo first and second order equations. The maximum biosorption capacity achieved by*Bacillus subtilis* for cadmium metal was found 51.8 mg/g. Hence, this study suggested that *Bacillus subtilis* proved to be an effective biosorbent for the mitigation of cadmium ions from aquatic solutions.

Introduction:-

Pollution caused by heavy metal ions in aquatic ecosystems arises as an alarming situation for the whole world. Over the years, overexploitation of water has been elevated immensely due to industrialization, urbanization and incessant rise of the population which cause uncontrolled discharge of toxic pollutants in natural water bodies. These heavy metal ions lead to deterioration and contamination of the environment and also show detrimental effects on the health of humans and other life forms. "Heavy Metals" are the natural metallic elements that have remarkably high density and they cannot be degenerated due to their tenacious and persistent nature and are found toxic even at very low concentrations (Lenntech, 2004). Heavy metals namely cadmium, arsenic, chromium and lead are released into the environment during mining, smelting and other industrial processes. Cadmium is considered a highly toxic and carcinogenic metal and the major sources of its discharge are painting paper, fuels, photographic materials and electroplating industries (Gupta et al.,2004). In order to make the environment healthier for living beings, contaminated water bodies need to be rectified by developing new approaches for the remediation of wastewater. Several conventional methods are available to expel metallic ions from aquatic solutions namely chemical

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precipitation, membrane separation, coagulation and reverse osmosis (Kratochvil and Volesky, 1998) but these methods are highly expensive and release a huge mass of toxic sludge, which is quite tough to maintain(Zouboulis et al., 2004). Accordingly, the use of biological biosorbents for the treatment of wastewater received attention worldwide and owing to certain advantages involving environmental welfare and cost-effectiveness. Therefore, Bioremediation is an alternative for the expulsion of ions from wastewater, it is the mechanism that involves breaking down hazardous substances into nontoxic forms by utilizing biological agents such as microorganisms, bacteria, fungi, green plants (Sivasubramanian, 2006). These biological agents have an enormous potential for accumulating and decontaminating polluted solutions (Volesky, 1986).

Biosorption is the process that involves adsorption of heavy metal ions from wastewater by biological agents through metabolic mediated uptake or passive uptake (Fourest and Roux, 1992). It consists of a solid phase (biosorbent) and the other is the liquid phase (solvent) which holds metal ions that need to be adsorbed. Bacteria are used as suitable biosorbents due to their very tiny size, ubiquitous nature, and ability to grow in favorable environments and they have an immense binding ability towards metal due to the presence of diverse functional groups like amino, carboxyl and hydroxyl groups and they evolved various tolerant mechanisms including sorption, ion exchange and complexation against a broad range of varying environmental conditions (Wang and Chen, 2009).Heavy metal uptake has been tested by various bacterial isolates likely *Bacillus*, *Micrococcus*, *Escherichia*etc (Duda-Chodak et al., 2013).The elimination of lead ions from wastewaters by utilizing the bacteria *Pseudomonas*,*Bacilluscirculans* has been studied by a few authors (Azza et al., 2009). The efficiency of some bacteria like *Pseudomonas aeruginosa*, *Penicillium chrysogenum*, *Bacillussphaericus*have checked for the expulsion of Cr, Ni, Pb and Cd pollutants (Alvarez et al., 1999). Dead biomass of several bacterial strains (*Citrobacter*, *GeoBacillus*, *Klebsiella*, *Bacillus* sp.,) areused for the retention of metallic ions from contaminated medium (Puranik and Paknikar, 1999., Tunali et al., 2006). The bacterial metal uptake capacities are mainly found in a range of 568 to 0.70 mg/g (Wang and Chen, 2009).

Bacillus subtilis is a rod-shaped gram-positive bacterium and have immense potential to bind with the metal due presence of teichoic acids and their different cell wall structures (Colak et al., 2011). *B. subtilis*are common and easily available bacteria and few authors have reported its adsorption capacity towards Pb(II) metal(Bai et al., 2014). Dead biomass is more suitable compared to live biomass due to many ways including easy storage, large volumes of contaminated water may be treated, less time required to reach equilibrium, no discharge of harmful by-products and does not need a continuous supply of nutrients for metal adsorption (Hemambika et al., 2011).The potential of any biomass is mainly affected by various factors such as biomass, pH of the medium, contact time and interaction with other metal ions.Equilibrium data arewell described by Freundlich and Langmuir adsorption isotherms which provide the relationship between biomass/sorbent and adsorbate for the sorption process(Muhamad et al., 1998). Any more metal removal through bacterial biomass increasing attention worldwide and its utilization for remediation of contaminated environment. Thus, the aim of this study is to evaluate the adsorption potential of the *Bacillus subtilis*dead biomass for common contaminant cadmium from aquatic solutions and the batch experiments were also performed to test the effects of various operational parameters such as the metal ion concentration of the medium, adsorbent dosage, contact time and pH of the solution on adsorption. The equilibrium and chemical kinetics data of the adsorption process were also studied by applying (Langmuir and Freundlich)and (pseudo first and second order) models.

Materials and Methods:-

The Microorganism: -

Bacillus subtilis (NCIM 2063)is a gram-positive bacteriumthat was procured from the National Collection of Industrial Microorganisms (NCIM), Pune in a freeze-dried culture, stored at -20°Cand standard techniques were used for inoculation of cultures.

Bacterial Identification and Characterization: -

The selected bacterial isolate was identified in accordance with the keys of Bergey's Manual of Systematic Bacteriology 2010 edition (Holt et al., 1994). These tests involve gram staining, motility and biochemical tests like indole, vogesproskauer, citrate utilization, nitrate reduction, urease etc.

16S rRNA Gene Sequencing: -

Bacillus subtilis (NCIM 2063) strain identification was confirmed by performing 16S rRNA gene sequence analysis which is an extensively accepted tool for molecular identification and resemblance against the Gene Bank database

(Kolbert and Persing, 1999). To determine the 16S rRNA gene sequence of the isolate, cells were lysed and the fragment was amplified by PCR utilizing the following primers:530F (5’ ATGTGAAAGCCCCGGGGAACG 3’) and 907R (5’ GCTCAGGACGAACGCTGG 3’) 1440bp (Darby et al., 2005).Basic Local Alignment Search Tool (BLAST) analysis with nearest culture sequence reclaimed from the National Centre for Biotechnology Information (NCBI) database that finds regions of local similarity between sequences (Altschul et al., 1990). A phylogenetic tree was constructed by carrying out the multiple alignments among selected bacterium 16S rRNA gene sequences of the database (Tamura et al., 2013).

Chemicals and Reagents: -
In this work $CdCl_2$ (Merck 208299) metal salt was taken as the respective source of cadmium metal ions and then1000 mg/L stock solution of cadmium was prepared by dissolving the 1.63 gm of $CdCl_2$ in 1000 ml of distilled water and the stock is diluted further to get desired metal concentrations (20, 40 60, 80, 100 mg/L).

Instrumentation: -
Atomic Absorption Spectrophotometer (Perkin Elmer AAnalyst 100, USA) was utilized for heavy metal estimation. Measurements of pH were done by utilizing a digital pH meter (HI96107, Hanna Instruments). Biosorption experiments were carried out in (Incubator shaker, Metrex scientific instruments, Delhi) and dead biomass of biosorbent was obtained in (Hot air oven, Universal, India). Falcon centrifuge tubes were obtained from (Thomas Scientific, India) and filtration of water samples was done by utilizing a 0.45μm membrane filter paper.

Bacterial Biosorbent (Biomass) Preparation: -
First of all, preparation and sterilization of nutrient medium were done and a loop containing bacterial culture was taken and then streaked on the agar plate in order to get more colonies.The nutrient broth was prepared for subculturing of*Bacillus subtilis*and then sterilized media (200 ml) was shifted to the Erlenmeyer flask. Under a laminar airflow chamber, the media was allowed to cool and then 200μl bacterial solution was inoculated in the medium. After inoculation, flasks were kept for incubation in an incubator shaker at 200 rpm speed at 30°C for two days to acquire the biomass. In order to get the desired biomass centrifugation was done at 9000 rpm for ten minutes. The supernatant was then discarded and the cell pellet was suspended again in distilled water to assure that no media was left on the surface of the cell. The cell pellet was centrifuged again and then the pellet was kept in a hot air oven at 90 °C for 12 hrs.to acquire dead (heat-killed) cells. The obtained dead biomass was used for subsequent biosorption studies.

Figure 1:- Biomass of *Bacillus subtilis*.

Biosorption studies: -

Figure 2:- Biosorption experiment design at various pH and Biomass ranges.

In this study, selected bacteria were assessed for uptake of toxic metal cadmium. Heat-killed dead biomass of*Bacillus subtilis*was used for the adsorption experiments and the capability of dead*Bacillus subtilis*in heavy metal uptake was determined *in vitro* conditions at various parameters namely a) pHb) Biomass dosage c) Temperatured) Contact timee) Initial metal concentration. Sorption experiments were conducted by an equilibrating adequate amount of sorbent biomass within100 ml of solutions of the desired cadmium metal concentration in 250 ml Erlenmeyer flasks. The flasks were kept on a rotary shaker at the speed of 200 rpm allowed to attain equilibrium. The samples were then centrifuged and the supernatant was analyzed to measure residual metal concentration by AAS.

pH Optimization: -

To study the effect of pH on the metal adsorption in the range(1 to 8)the optimum amount of biomass was dipped in 100 ml of the solution containing 20 mg/L of Cd metal concentration and then the flasks were left to attain equilibrium. HCL and NaOH were used as pH regulators for adjusting the solutions of pH. After the centrifugation and filtration, the supernatant was collected to analyse the residual metal concentration.

Biosorbent Dose Optimization: -

To determine the influence of biomass dose, experiments were carried out at different doses of biomass (1 mg/ml to 4 mg/ml) in the presence of optimum ranges of other parameters with a 100 ml solution of 20 mg/L of cadmium metal concentration. The flasks were kept on a shaker to attain equilibrium and then samples were collected for the analysis of the concentration of metal.

Temperature Optimization: -

Experiments were carried out to monitor the influence of temperature on adsorption at different temperature ranges (25-45^{0}C) having 20 mg/L metal concentration in 100 ml of solution at optimum ranges of pH and biomass.

Contact Time Optimization: -

An adequate amount of biomass (0.2 g) was dissolved in a solution of 100 ml containing 20 mg/L cadmium metal concentration and experiments were performed at optimum pH and biomass range, then the flasks were kept on a rotary shaker at 200 rpm speed to achieve equilibrium. The samples were collected at regular intervals of time (10 to 120 minutes) and then the supernatant was analyzed for the residual metal content.

Initial Metal Concentration Optimization: -

Experiments were performed at diverse initial metal concentration ranges (20 to 100 mg/L) of Cd metal by keeping the optimum ranges of all the other parameters obtained from previous experiments to attain equilibrium and the removal percentage was calculated by the initial and final metal concentrations of solution.

Data Evaluation: -

Adsorption Isotherms (Onyancha et al., 2008)

The Langmuir and Freundlich isotherms were applied to examine the adsorption data for the removal of Cd ions

andimpart information on the equilibrium among the adsorbed metal ions on the biomass surface and the remaining concentration of metal in the solution. The Langmuir and Freundlich model equations are given below:
Langmuir equation:

$$q_e = \frac{q_{max} b C_e}{(1 + bC_e)}$$

Freundlich equation:

$$q_e = K_F (C_e)^{1/n}$$

Where q_e is the metal adsorbed (mg metal per g biomass), q_{max} is the maximum amount of metal adsorbed (mg/g), b is the Langmuir constant related to the binding between the adsorbent and sorbate and K_F is a Freundlich constant, C_e is the equilibrium metal ion concentration in the solution (mg/l) and 1/n is adsorption intensity.
Before data analysis for the Langmuir and Freundlich isotherms, the amount of metal bound to the adsorbents (q_e) and the percentage biosorption of cadmium metal (R) were calculated as follows:

$$q_e = \frac{(C_0 - C_e)}{m} xv$$

$$R(\%) = \frac{(C_0 - C_e)}{C_0} x100$$

Whereq_e is the metal adsorbed(mg/g), $C_0$and $C_e$are the initial and final metal concentrations (mg/l) respectively, v is the volume of solution (ml), and m is the biomass of *Bacillus subtilis*biosorbent (mg).

Chemical Kinetics (Yang and Duri, 2005)
The chemical kinetics applied for metal sorption by two united kinetic models.

a) Pseudo first-order kinetic equation
b) Pseudo second-order kinetic equation

Kinetic models namely pseudo-first-order and pseudo-second-order describe that sorption is a pseudo-chemical reaction and sorption rate can be studied by the first and second-order reactions respectively. The rate equations are presented below:

$$\frac{dq_t}{d_t} = k_1(q_e - q_t)$$

$$\frac{dq_t}{d_t} = k_2(q_e - q_t)^2$$

Where (q_e)mg/g is the metal adsorbed at equilibrium, (q_t) mg/g is the metal adsorbed at time (t) minutes, and (k_1) minutes^{-1} and (k_2)g mg^{-1} minutes^{-1} are the rate constant for pseudo-first-order and pseudo-second-order respectively.

Results and Discussion: -

Biochemical Characterization of Isolate: -

Biochemical characterization of isolates was done on the basis of Bergey's Manual of Determinative Bacteriology (as given in Table 1).

Table 1:- (+) and (-) signs indicate positive and negative reactions, respectively.

S.No.	Biochemical Test	*Bacillus subtilis*
1	Urease	-
2	Glucose	-
3	H_2S	-
4	Citrate	+
5	Indole	-
6	Lactose	-
7	Methyl Red	-
8	Oxidase	-

9	Catalase	+
10	V.P Test	+

Molecular Identification (by 16S rRNA Gene Sequencing): -

Identification of bacterial isolate NCIM 2063 was confirmed by16S rRNA gene sequencing which showed 99 % resembles with *Bacillus* species (Closer to halotolerans) when analysed by NCBI blast (National Center for Biotechnology Information -Basic Local Alignment Search Tool) and NCIM 2063 was further identified by Vitek 2 (VT291020) and confirmed as *B. subtilits*strain.Evolutionary analyses were done in MEGA6 (Tamura et al., 2013).

Phylogenetic Analysis: -

Phylogenetic trees as shown in (Figure 3) were constructed with the Neighbor-Joining method with the sum of branch length of 0.07064957 for NCIM 2063 and phylogenetic analysis of NCIM 2063 involved 19 nucleotide sequences. There were a total of 1404 positions in the final dataset for the isolate *Bacillus subtilis*.

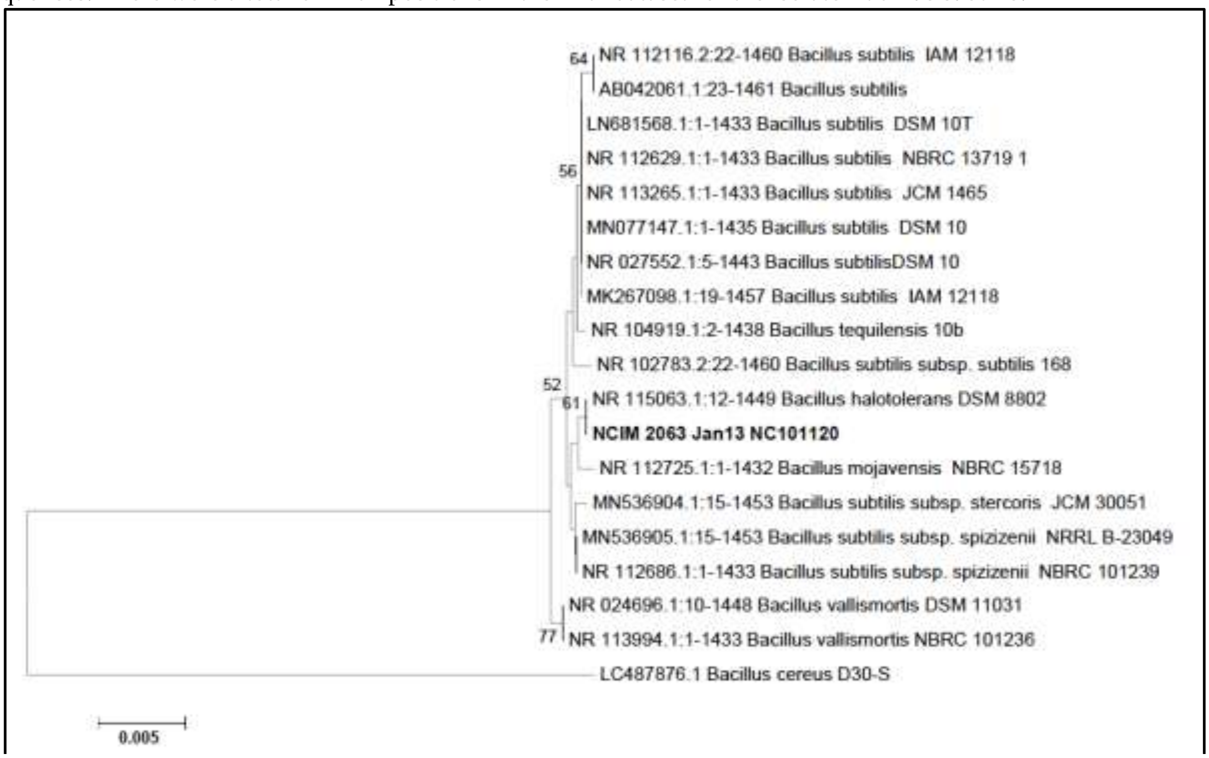

Figure 3:- Neighbor-joining phylogenetic tree based on 16S rRNA gene sequencing of *Bacillus* sp.Copyright @ NCIM, CSIR-NCL.

Biosorption Experiments: -

In the present investigation, the dead biomass of *Bacillus subtilis*was taken for the adsorption of cadmium metal ions. The operational parameters influencing the cadmium adsorption by using *Bacillus subtilis* biomass were determined. Effects of different parameters are given as follows:

Effect of pH: -

One of the most notable factors is the pH of the medium which influences nearly all biological and chemical reactions. Biosorption studies were performed at a pH range of 1 to 8 and in batch experiments, the pH of the medium affects intensely the sorption of the metallic ions on the surface of the selected adsorbent. Results inferred that the highest adsorption was found at pH 5 and the adsorption percentage increased from 40.81% at pH 1 to 87.50 % at pH 5 and then shows a slight decline with the rise in pH range. The trend of pH in these experiments is given in (Figure 4), from the present study it can be reported that at pH 5, *Bacillus subtilis* exhibitedthe highest percentage of adsorption.

Similar results were observed by (Vijayaraghavan and Yun, 2008) who reported that the decline of removal percent at higher pH ranges may be because of the low solubility of metal complexes, which adequately allowing precipitation and may convolute the adsorption process. Similar results were also reported, for the remediation of metallic ions by bacterial biomass (Esposito et al., 2001). Wang and Chen (2009) observed similar results for *Bacillus, Pseudomonas*and *Micrococcus*species that they have the capability to adsorb maximum Cu, Cd, and Pb at moderate pH not very acidic nor very basic. However, with the rise of pH, negative charges ligand also increases and can easily bind with the cations (Ahuja et al., 1999).

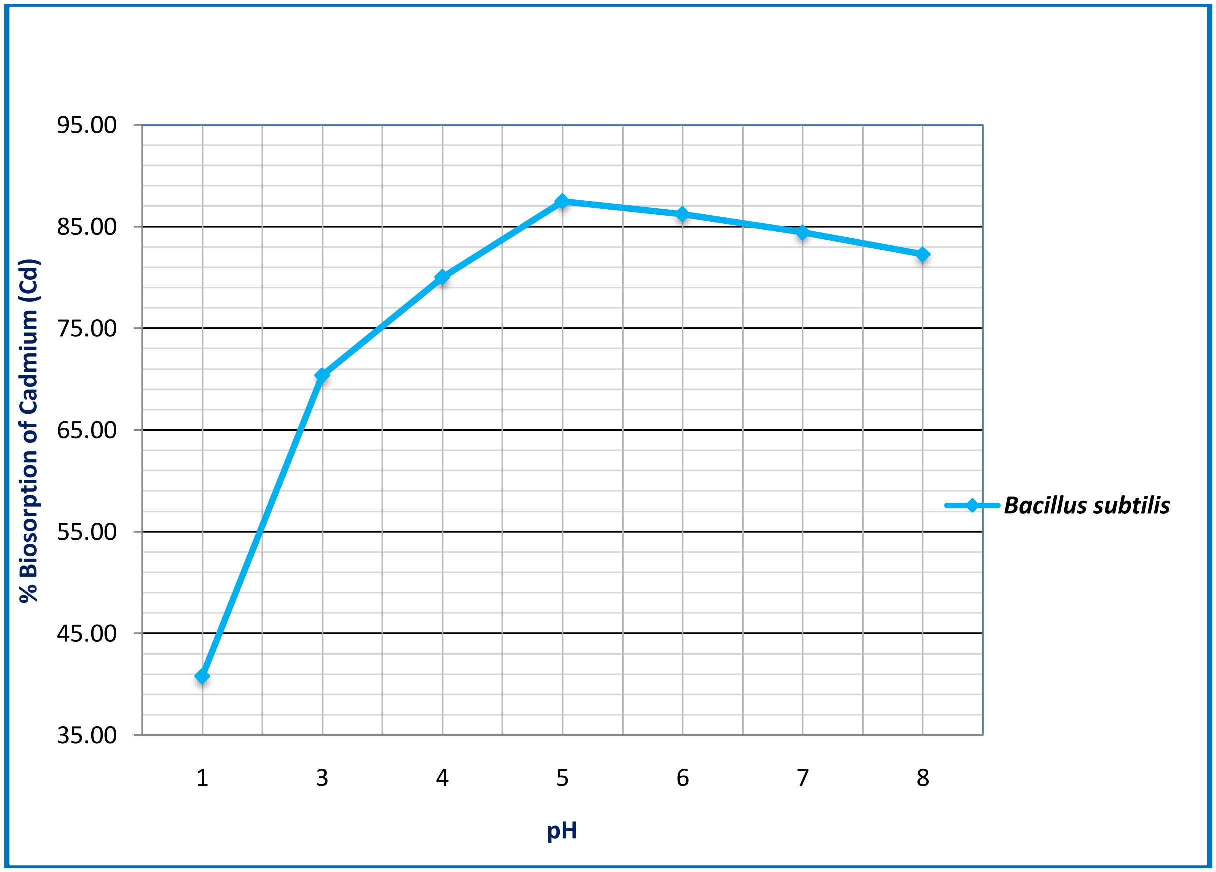

Figure 4:- Effect of pH on Biosorption of Cadmium onto *Bacillus subtilis*.

Effect of Biomass Dosage: -

Adsorption of cadmium was conducted at different biomass dosages of dead biomass of *Bacillus subtilis*. In order to attain the highest adsorption capacity of the selected sorbent for cadmium, the experiments were conducted at varying ranges of biomass dosages from 1 to 4 mg/ml. Results inferred that 200 mg biomass amount in 100 ml solution was found adequate for the maximum cadmium adsorption, beyond this it was reported nearly constant. From the above experimental data, it has been concluded that biomass is a significant factor to be considered for adsorption. The unnecessarily increased biomass does not require metal removal. After a certain limit, biomass did not affect the adsorption rate much. This might be due to the absence of binding sites to the metallic ions and maybe also due to the obstruction of binding sites with excessive biomass. In many instances, low biosorbent dosages result in higher uptakes. (Aksu and Cagatay, 2006; Vijayaraghavan et al., 2006) and generally, with the rise of biomass dose,the amount of solute sorbed also increases due to the expansion of the surface area of the adsorbent, which allows an increase in the number of attaching sites (Esposito et al., 2001).

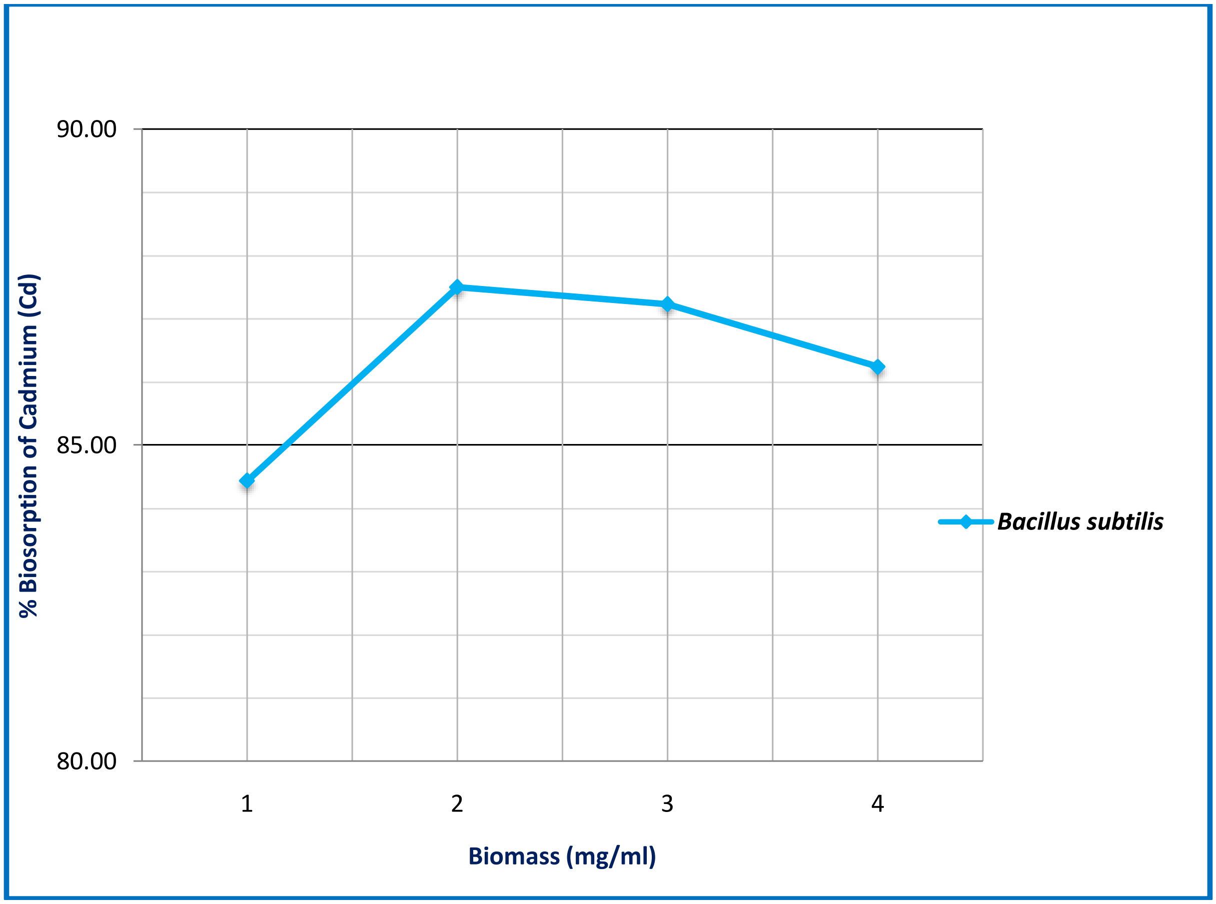

Figure 5:- Effect of biomass amount on Biosorption of Cadmium onto *Bacillus subtilis*.

Effect of Temperature: -

The effect of temperature on cadmium adsorption is given in(Figure 6) and adsorption experiments were carried out at different ranges of temperatures 25^{0}C to 45^{0}C. Results showed that the highest adsorption percentage around 87.50 % was reported in the temperature range of 25^{0}C. In the performed experiments there was a slight decline in adsorption percentage was recorded with an increase in the range of temperature from 25^{0}C to 45^{0}C. This possibly results in the shrinkage of cells at high ranges of temperatures which can cause a reduction of the surface area of contact. The temperature within the range from 20 to 35 °C, influences the adsorption mechanism only to a lesser extent (Vegliòand Beolchini, 1997). Moreover, at higher ranges of temperatures adsorbent physical damage can be expected because of the exothermic nature of some adsorption mechanisms and in many cases, the efficiency of biomass reduces, with the riseintemperature range (Mameri et al., 1999). The temperature parameter was considered an important factor during the biosorption process (Suhasini et al., 1999). The influence of temperature on the biosorption of metal ions on *B. subtilis*biomass was investigated, the removal rate was increased when the temperature increased from 15 to 35^{0}C. Thus, in order to save energy, 35^{0}C temperature is found optimum for biosorption experiments (Cai et al., 2018).

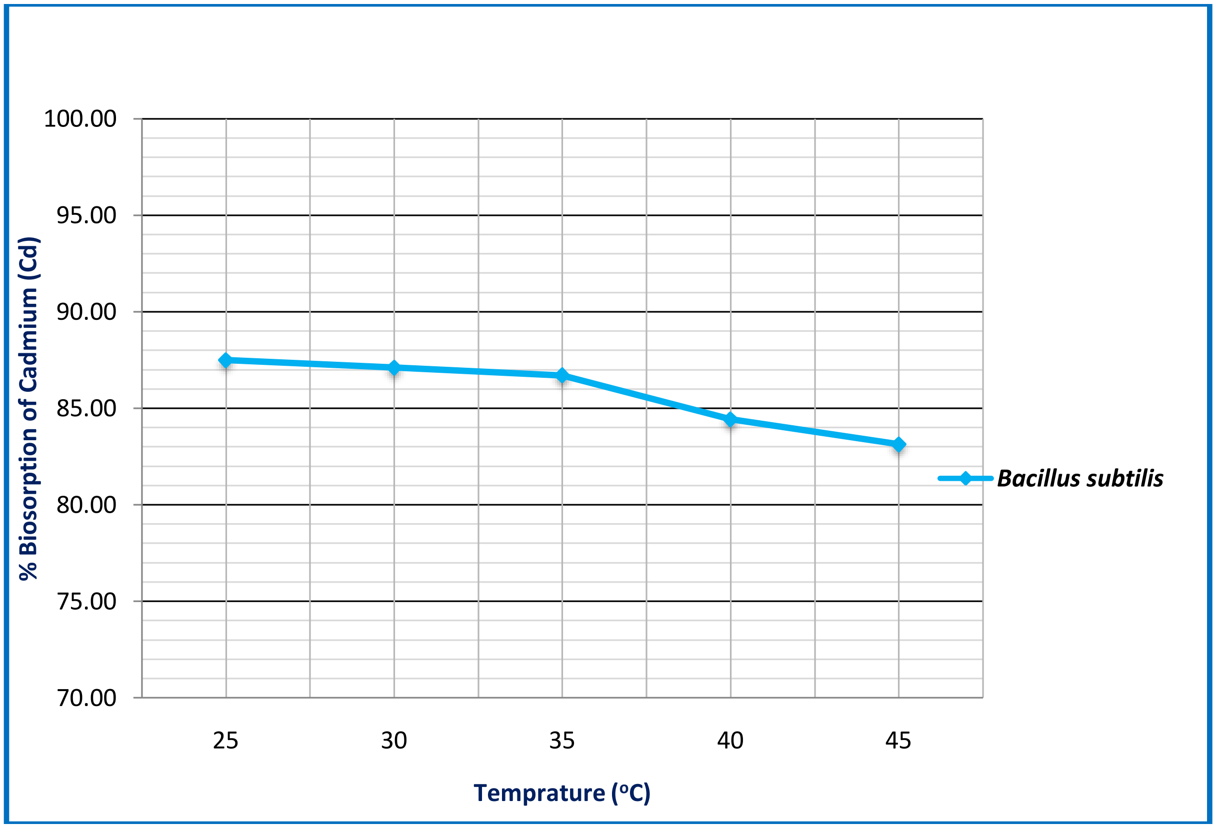

Figure 6:- Effect of temperature on Biosorption of Cadmium onto *Bacillus subtilis*.

Effect of Contact Time: -

Contact time considered a crucial factor for optimization. The optimum amount of biomass was dipped in the solution and the equilibrium time at which the highest sorption took place was evaluated. The sorption studies of cadmium were carried out at different time intervals by keeping other parameters constant (adsorbent amount of 0.2 g at pH 5 and 25^0C). Results are given in (Figure 7) indicated that the adsorption rate of cadmium metal increased with the rise in contact time till equilibrium is attained and a further increase in time did not influence the removal rate and it was found nearly constant. More than 50 % of cadmium ions retention was completed in the first 10 minutes and the maximum biosorption percentage (87.50 %) of cadmium occurred at 60 min of equilibrium time.This short time required for the sorption process is in accordance with the result given by others (Chen et al., 2005;Tsezos and Volesky, 1982), the equilibrium time for lead were 40 and 50 minutes at *Bacilluscereus* and *Bacillus subtilis* respectively. Similar kinds of results have been also discussed by (Gabret al., 2008) for metal removal. The impact of contact time on the adsorption of metal ions on the biomass of *Bacillussubtilis*and the sufficient time for adsorption process was found 45 min, where the highest sorption was seen and afterward, there was no considerable rise was noticed. Hence, the adsorption of heavy metal ions was very fast in the initial stages of the experiment because a huge number of unoccupied sites were present on the surface of biomass (Colak et al., 2011).

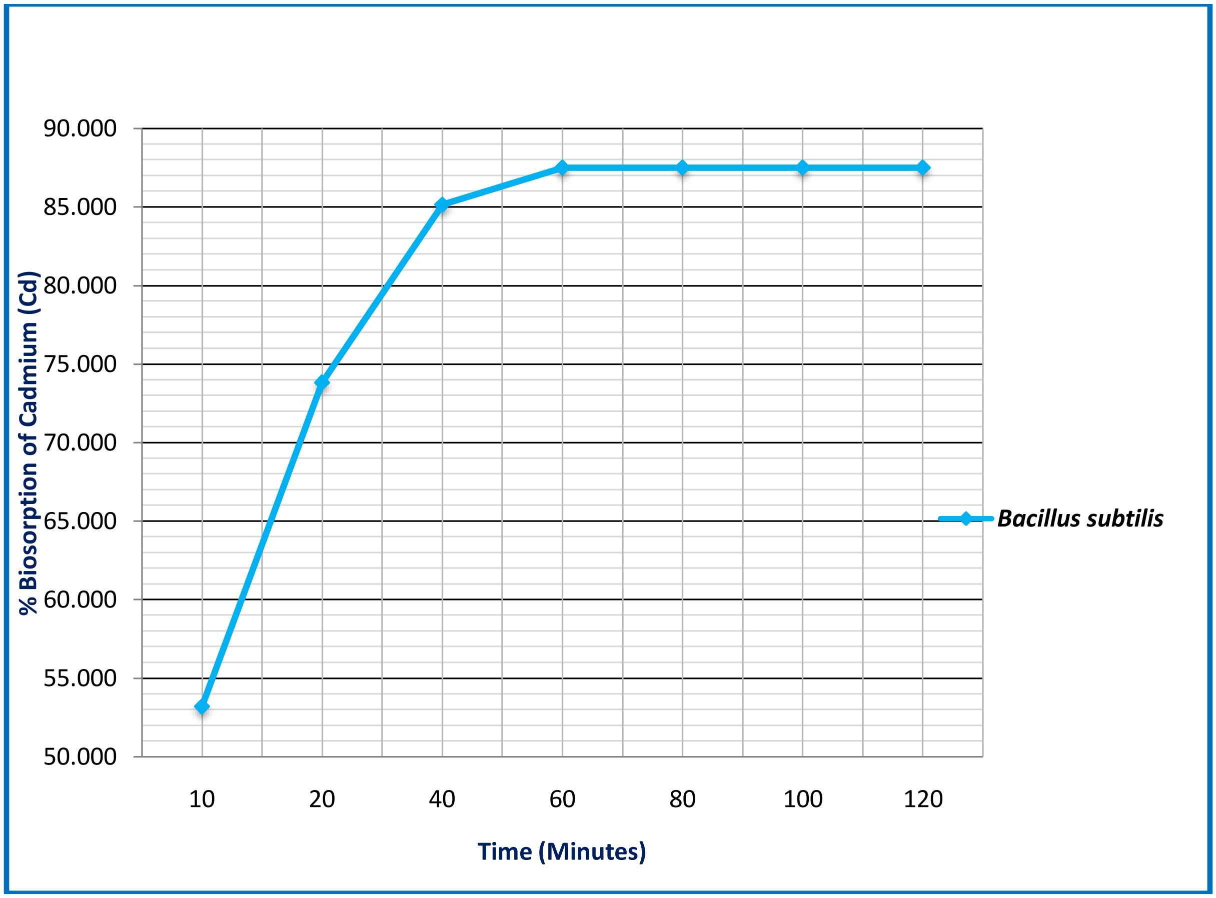

Figure 7:- Effect of contact time on Biosorption of Cadmiumonto *Bacillus subtilis*.

Effect of Metal Concentration: -

The impact of initial metal concentration was determined by varying the concentration range of cadmium from 20 to 100 mg/L in the presence of an optimum range of other factors. Results illustrated in Figure 8; when the cadmium metal concentration was increased from 20 to 100 mg/L, the removal percentage of cadmium by*Bacillus subtilis* dead biomass decreased sharply from 87.50 % to 72.50 % respectively. Therefore, it can be suggested that with the rise in metal concentration, the percentage of biosorption decreases. Nevertheless, the best metal ions retention on considered biosorbent was occurred at the lowest value of initial metal concentration (20 mg/L).Similar studies have been shown by (Sabae et al., 2016) that the adsorption rate declines with the rise in the concentration of metal ions. At higher concentrations, a lower adsorption yield was noticed resulting in the early saturation of binding sites for the adsorption process (Kadukova and Vircikova, 2005; Pandiyan and Mahendradas, 2011). Similar studies were performed for chromium and the highest removal occurred at a minimum metal concentration by *Bacillus*species (Salehizadesh and Shojaosadati, 2003).

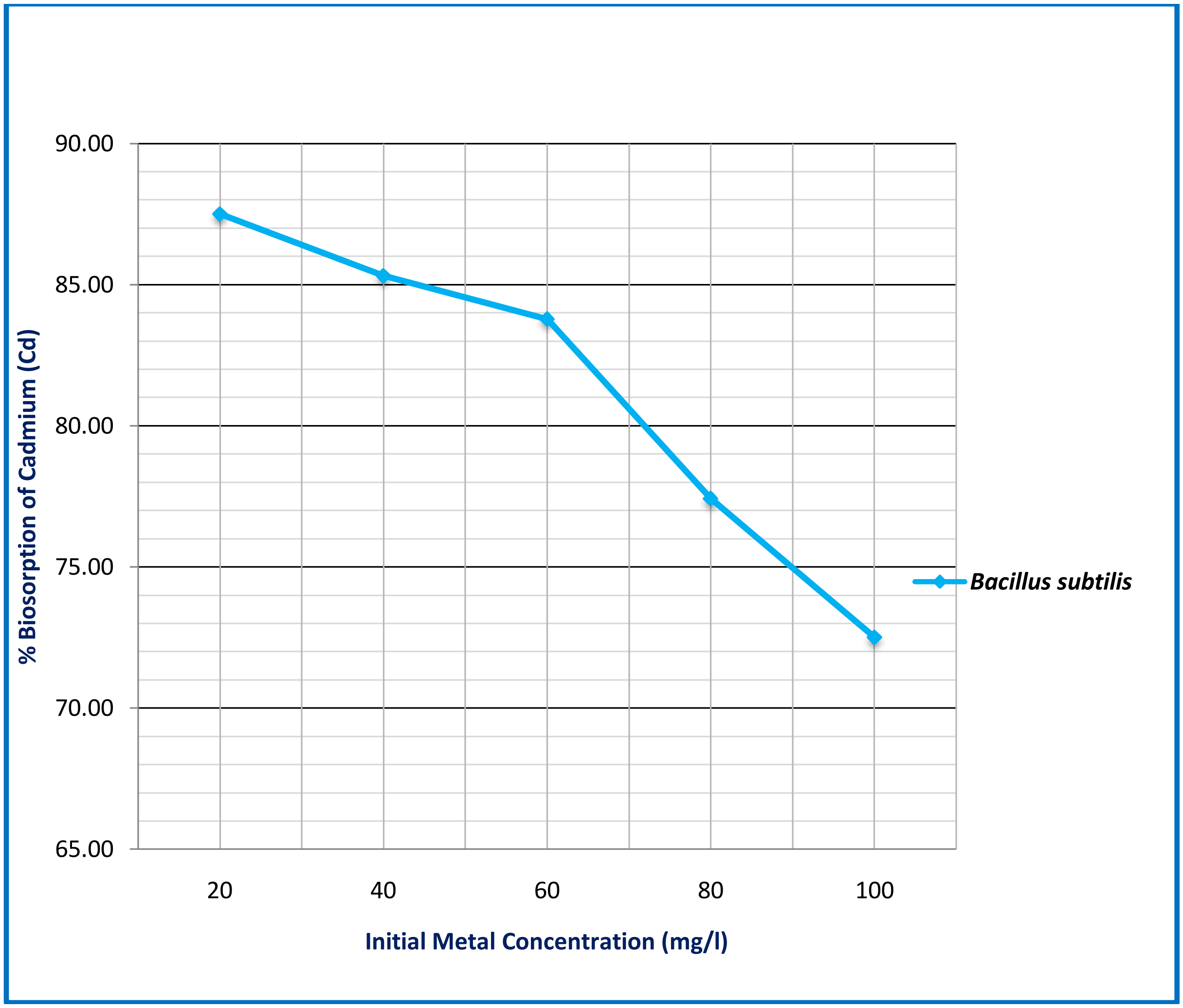

Figure 8:- Effect of initial metal concentration on Biosorption of Cadmium onto *Bacillus subtilis*.

Chemical Kinetics: -

Suitable kinetic models were applied for the adsorption of cadmium for dead biomass of *Bacillus subtilis*biosorbent. The data were analyzed by the kinetics first-order and second-order equations. The equation of first order was plotted for ln (q_e-q_t) against t is given in Figure 9 (a) and the equation of second order was plotted for t/q_t against t is shown in Figure 9 (b). The correlation coefficient for pseudo-first-order and pseudo-second-order was reported around R^2= 0.9999 and R^2= 0.9986 respectively. Kinetic equations imply that the pseudo-first order equation was well fitted with cadmium adsorption than the pseudo-second order equation for the selected bacterial isolate. Results related to chemical kinetics data are given in Table 2.Experimental data were used to determine the sorption process and its sorption rate-controlling step that involves mass transport and chemical reaction (Mehmet and Sukru, 2006). The chemical kinetics involves the metal expulsion rate that manages the contact time of the adsorbate in the interface of the solution. Kinetic models are used to explain the liquid-solid phase relationship in adsorption mechanisms (Kosa et al., 2012).

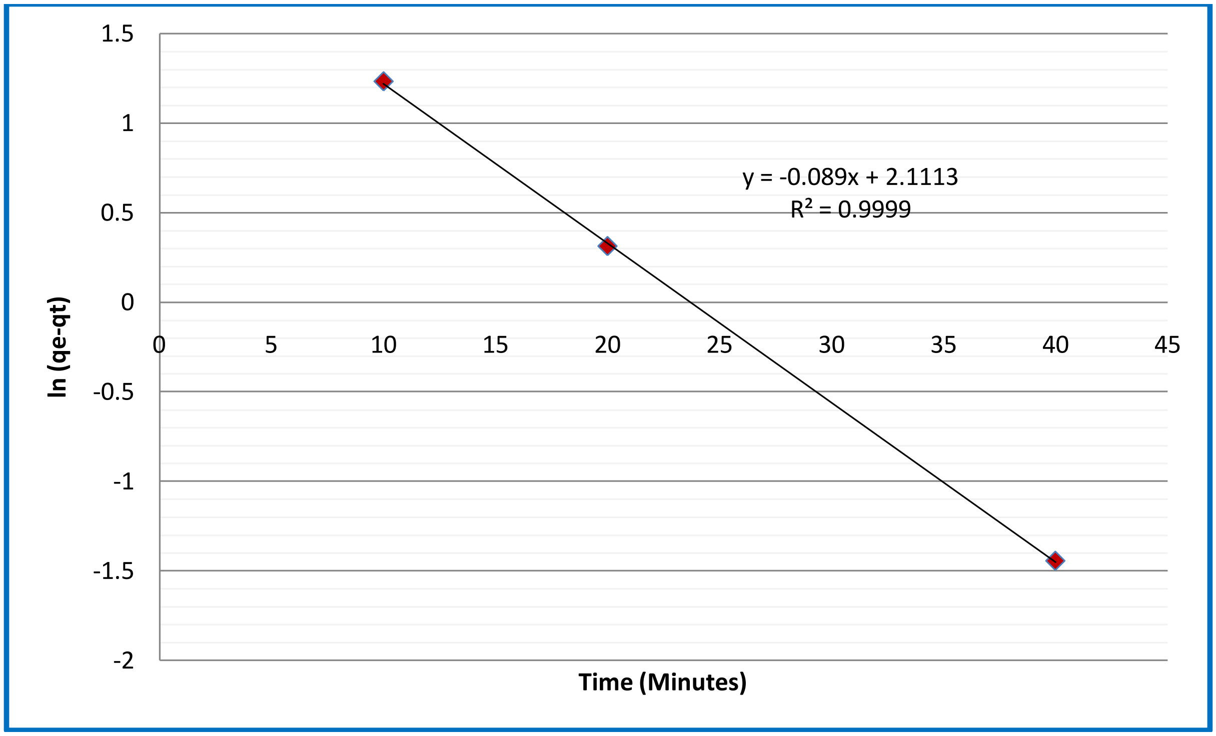

Figure 9 (a):- First order kinetics for Cadmium biosorption onto *Bacillus subtilis* dead biomass

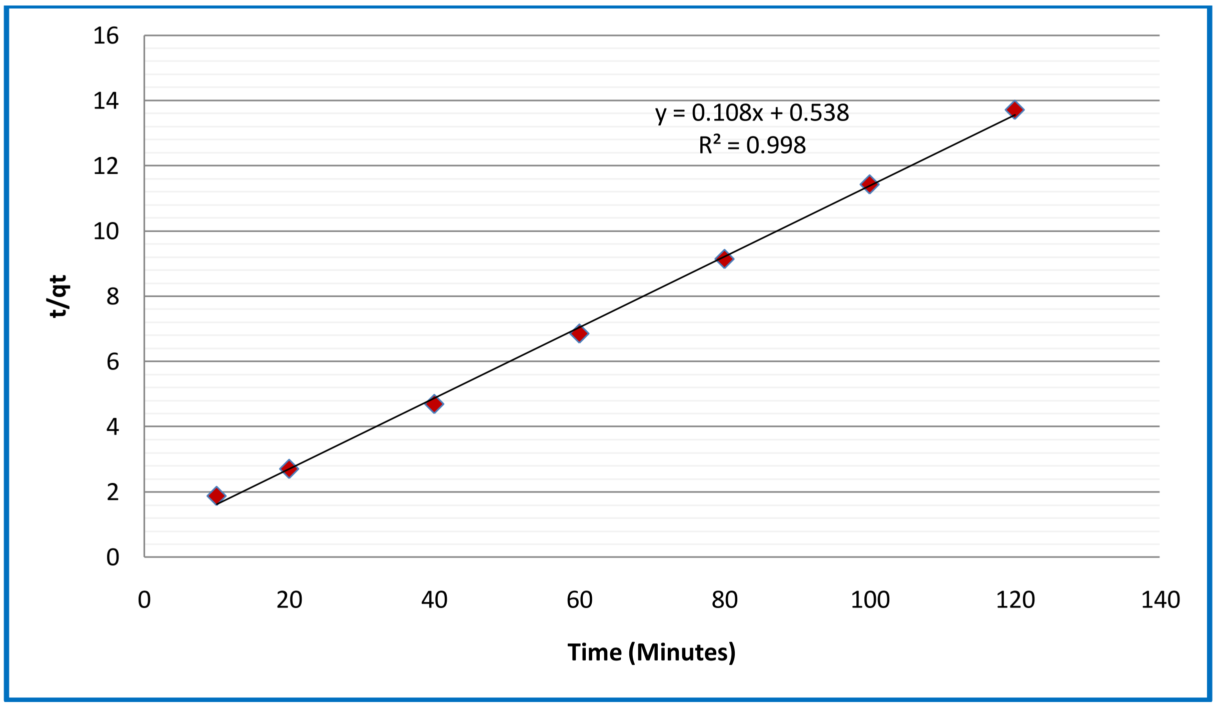

Figure 9 (b):- Second order kinetics for Cadmium biosorption onto *Bacillus subtilis* dead biomass.

Table 2:- Kinetic data of dead *Bacillus subtilis* for biosorption of Cadmium.

Kinetic Data of *Bacillus subtilis* for Cadmium (Cd)						
Heavy Metal	Pseudo First Order			Pseudo Second Order		
Cadmium (Cd)	**K_1**	**q_e**	**R^2**	**K_2**	**q_e**	**R^2**
	0.089	8.258971	0.9999	0.021873	9.21659	0.9986

Adsorption Isotherms:-

Langmuir and Freundlich isotherms were investigated for the adsorption of cadmium by dead biomass of *Bacillus subtilis*. The experimental results of the Langmuir model are depicted in Figures 10 (a) and the Freundlich models are shown in Figure 10 (b). For both the models the coefficient of determination (R^2) was found more than 0.95 and nearly close to 1. This implies that both isotherms appropriately describe the adsorption data for the cadmium. In Table-3 summarized linear regression data were studied for Langmuir and Freundlich models for cadmium adsorption by using dead biomass of bacterial isolate and K_F, n, q_{max} and b values were also estimated. Adsorption data suggested that for the cadmium metal, Langmuir isotherm (R^2 = 0.9938) was better fitted in comparison to the Freundlich model (R^2 = 0.9642) and the maximum adsorption capacity (q_{max}) achieved by*Bacillus subtilis* for cadmium metal was 51.8 mg/g.

Moreover, at greater concentrations, the metal ions need to move into the surface of biomass by intra particular diffusion process and the hydrolyzed ions may move at a very slower rate (Gabr et al., 2008; Sang et al., 2009). The overall observation from both postulated Langmuir and Freundlich isotherms for metals biosorption was in accordance with many studies (Pardo et al., 2003; Saret et al., 1999). The Langmuir and Freundlich isotherms are the most reported models used in biosorption processes by various authors, because they are simple, well-established models (Vijayaraghavan and Yun, 2008).

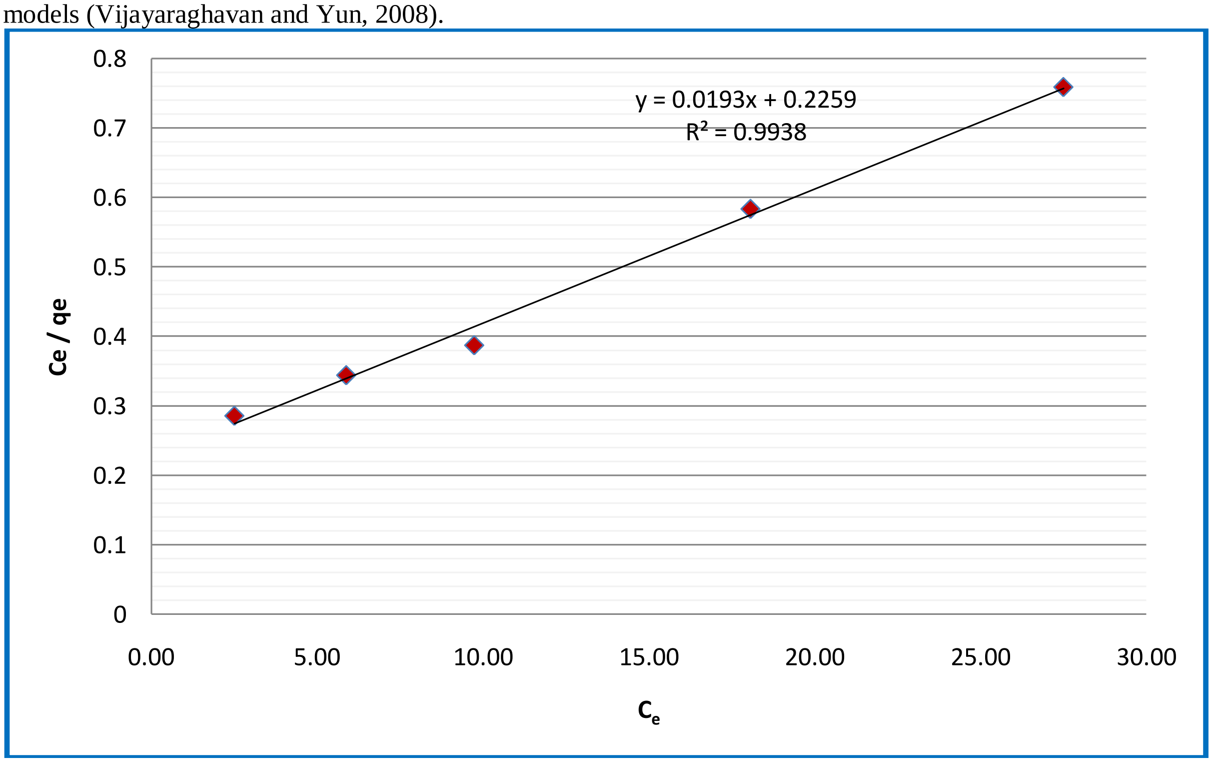

Figure 10 (a):- Adsorption isotherm (Langmuir) for Cadmium biosorption by using dead biomass of *Bacillus subtilis*.

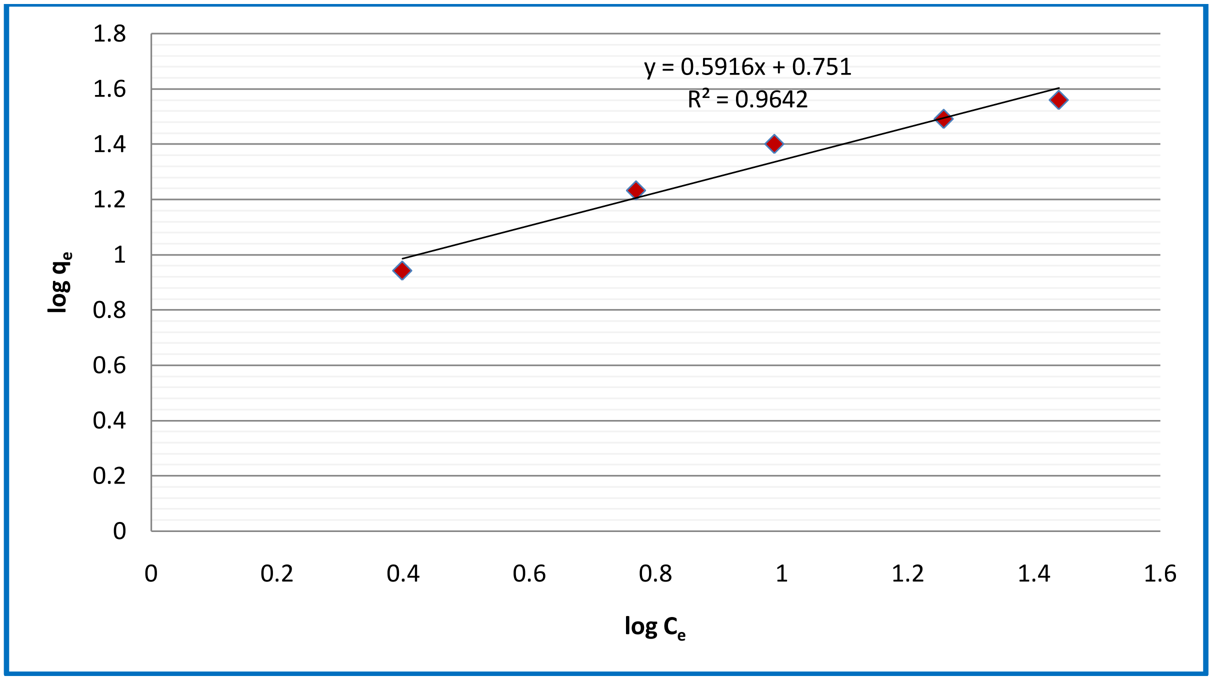

Figure 10 (b):- Adsorption isotherm (Freundlich) for Cadmium biosorption byusing dead biomass of *Bacillus subtilis*.

Table 3:- Parameters of isotherm (Langmiur and Freundlich)modelslinear representation for heavy metal Cadmium onto *Bacillus subtilis* dead biomass.

Adsorption Data of *Bacillus subtilis* for Cadmium (Cd)						
Heavy Metal	Langmuir Parameters			Freundlich Parameters		
Cadmium (Cd)	q_{max}	**b**	R^2	K_F	**1/n**	R^2
	51.81347	0.085436	0.9938	5.636377	0.5916	0.9642

Conclusions:-

This study gives relevant details about the suitability of *B. subtilits* biomass as a biosorbent for the removal of cadmium heavy metal. The batch experiments adsorption study shows that the process of adsorption is based on various parameters such as pH, biomass, contact time, metal concentration and the optimum ranges obtained for the maximum removal (87.50 %) of cadmium was achieved at pH 5, biosorbent dose 0.2 g, after 60 min of contact time from 100ml solution.The adsorption data were well fitted towards the Langmuir model (R^2 = 0.9938) than the Freundlich model and the kinetic data obtained for biosorption of metal followed the pseudo first-order more efficiently than second-order kinetic model. The adsorption capacity (q_{max}) was noticed to be 51.8 mg/g for cadmium metal.From the results, it can be inferred that *Bacillus subtilis*could be utilized as a suitable adsorbent for the treatment of cadmium containing heavy metals polluted water.

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