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Biosorption of heavy metals by using free and immobilized cells of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

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ABSTRACT

Various methods which are commonly used for the wastewater treatment for heavy metals, In the present work the free and immobilized cells of *Pseudomonas aeruginosa., Klebsiella pneumoniae*, were studied as biosorbent materials for the removal of Zn, Mg, and Ba from aqueous solution. . The both free cell and immobilized cells of *Pseudomonas aeruginosa* showed maximum sorption of heavy metals (Zn, Mg, and Ba) compared with *Klebsiella pneumoniae*. In this study % of sorption capability was calculated by using Gravimetric method. *Pseudomonas aeruginosa* has high ability of heavy metal sorption such as Zn, Mg, and Ba compared with *Klebsiella pneumoniae* sorption ability of Zn, Mg and Ba. The reaction mixtures were incubated with varied initial concentration of biomass to verify the change on the sorption capacity of the bacterial biomass. The biosorption concentration was increased simultaneously heavy metal sorption ability also increased when the biomass concentration increased. The different parameters like biomass concentration, pH, contact time and temperature were also analyzed. Hence these organisms are used for the wastewater treatment, which is a cheapest method, due to the biosorption capacity of the organisms. The biosorption performance of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* can be implemented practically for the treatment of wastewater.

KEYWORDS

Heavy metals;
Biosorption;
Zinc;
Magnesium and Barium.

INTRODUCTION

Natural waters have been found to be contaminated with several heavy metals, arising mostly from mining waste and industrial discharges[24]. These contaminants cause problem to the ecosystem. Although some heavy metals are necessary for the growth of plant after certain concentration, they become poisonous to both plants and microorganisms. Sometimes, these heavy metals are held in soil as a result of adsorption, chemical reaction and ion exchange[3]. Heavy metals of concern include lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, silver, gold and nickel[21]. The metals increase in concentration at every level of food chain and are passed onto the next higher level of phenomenon called bio-magnification[16].

Exposure to heavy metal contamination has been found to cause kidney damage, liver damage and anaemia in low doses and in high concentrations it can be carcinogenic and teratogenic if not fatal[2]. To avoid health hazards it is essential to remove this toxic heavy metal from waste water before its disposal. Main source of heavy metal contamination include urban industrial aerosols, solid wastes from animal mining activities industry and agricultural chemicals. Heavy metals also enter the water supply from acid rain which breakdown soils and rocks, releasing heavy metals in to streams, lakes, and ground water.

Various methods which are commonly used for the wastewater treatment for heavy metals, includes precipitation, ion exchange, adsorption, reverse osmosis, eletrodialysis, evaporation, liquid membrane techniques, solvent extraction and crystallization[11]. Immobilized non living biomass and metal binding compounds can also be used to remove heavy metals. Freely suspended microbial biomass has disadvantage that include small particle size and low mechanical strength. Immobilized bioreactor using *Bacillus coagulans* have been tested for Cr (VI) reduction. *Pseudomonas species, Streptomyces species,* and *Citrobactor species,* immobilized on PVC have been reported to be very effective in heavy metal removal, namely vandium, cadmium, copper, lead with high efficiency[23].

Biosorption involves the removal of metal or metalloid species, compounds and particulates from the solution by biological material[7]. Biosorption is a property of certain types of inactive, dead, active microbial biomass to bind and concentrate heavy metals from even very dilute aqueous solutions. In the present work the free and immobilized cells of *Pseudomonas aeruginosa., Klebsiella pneumoniae,* were studied as biosorbent materials for the removal of Zn, Mg, and Ba from aqueous solution.

EXPERIMENTAL

Study materials

The sample was collected from Kings chemical industry at Vadaseri, Thanjavur, Tamilnadu. The collected water sample was stored on specific aseptic container.

Isolation of bacteria[17]

Serial dilution was performed by using the collected water sample to isolate the organisms. From that 1ml of water sample was taken and diluted in to the tube containing 9ml of distilled water and mixed thoroughly, to make a 1:10 dilution (10^{-1}). Then 1ml of diluted sample was transferred to the next tube and serially diluted into the series of test tubes having 9ml of distilled water using sterile pipettes, up to 10^{-7} dilution.

Media preparation

Pseudomonas agar medium was prepared by using peptone – 20g, Glycerol– 10g, Dipottassium sulphate – 10g, Magnesium chloride – 3.5g, Agar- 15g were taken in 1000ml conical flask containing 1000 ml of distilled water and mixed thoroughly conical flask was plugged with cotton and sterilized in autoclave[5] The diluted 0.1 ml of sample was taken from 10^{-4} – 10^{-7} dilutions and were spreaded over the prepared Pseudomonas agar medium and nutrient agar medium then the plates were incubated at 37°C for 24 to 48 hours. After incubation the colonies were observed on the plates.

Identification of bacteria

The organisms were identified by Gram staining, Motility test[22] and Biochemical tests[13].

Microorganisms and culture condition

The biomass used for the biosorption of Zn, Mg, and Ba are *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were grown in 250ml conical flasks each containing 100ml separately . It consists of NaCl – 500mg, MgSO4 – 100mg, KCl – 100mg, Peptone – 0.6g, Sodium citrate – 1g, at a constant pH of 7.4.

The conical flasks were inoculated with freshly prepared broth culture suspensions and incubated at 37°C for one week under room temperature. The bacterial biomass were harvested by centrifugation at 4,000 rpm for 20 minutes, washed thoroughly with distilled water and then used for the biosorption studies.

Metal solution

The stock solution of Zinc, Magnesium, and Barium were prepared by dissolving 1g/L of $ZnCl_2$, $MgCl_2$ and $BaCl_2$ respectively in 15% NaCl solution prepared in distilled water. The exact dilutions of the respective stock solution were used for the biosorption studies (Plate-3).

Methods of immobilization

Pseudomonas aeruginosa and *Klebsiella pneumoniae* cells were im mobilized by sodium alginate. Entrapment of cells in non-toxic alginate is one of the simplest, cheapest and most frequently used for immobilization[20]. Sodium alginate and Calcium Chloride were used to prepare the alginate beads containing the whole cells. Sodium alginate solution (6% was prepared by dissolving 6gm of sodium alginate in 100ml distilled water. The contents were stirred vigorously for 10 minute to obtain thick uniform slurry without any undisclosed lumps and then sterilized by autoclaving. Both alginate slurry cell suspensions were mixed and stirred for 10 minutes to obtain uniform mixtures. The slurry was taken in to a sterile syringe, added drop wise in to 2% $CaCl_2$ solutions to get approximately 500 immobilized cell composites. Approximately 100 composites were used for the sorption studies.

Biomass characterization

The phosphates present on the cell wall of biomass were determined in order to study the nature and capacity of the biosorbent. The phosphate content was estimated spectrophotometrically by using Molybdenium blue method[10]. 50ml of sample was taken in clean conical flask. One drop of phenolphthalein indicator was added to it. Then 5N H_2So_4 was added along with. 8ml of mixed reagent (the reagent was prepared by mixing 50 ml of 5N H_2SO_4 with 5ml of potassium antimonyl tartarate reagent (100mg/50ml distilled water). To this 15ml of ammonium molybdate solution (2g/50ml distilled water) and 30ml of ascorbic acid solution (0.88g/50ml distilled water) were added and mixed thoroughly). The above reagents were mixed well and incubated at a room temperature for 10min. The absorbance was read at 680 nm in a spectrophotometer using distilled water with other reagent as blank.

Methods for biosorption studies

10µl of bacterial biomass was suspended in 10ml of metal solution is 100ml of conical flask and the flasks were incubated at 35°C. Samples withdrawn at definite intervals were centrifuged at 4000 rpm for 20min and the supernatant was analyzed for residual metal content. The residual metal content was determined by using gravimetric method.

Gravimetric estimation[18]

10ml of reaction solution was centrifuged at 4000 rpm for 20min. To 5ml of supernatant solution, 5ml of 10% precipitating agents were added. The resultant solutions were centrifuged at 4000 rpm for 20 minutes. Then the precipitates were collected, dried in a hot air oven and weighed in an electronic balance w_f. The same procedure was repeated with the standard metal (10 mg/ 10ml) solution to get the initial weight of metal, W_1.

Calculation

$$\%\ \text{of sorption}\ \frac{(w_1 - w_f) \times 100}{W_1}$$

1. 10% solution of Na_2HPo_4 and $(NH_4)_2\ SO_4$ were used as precipitating agent in the case of Mg and Ba respectively.
2. 10% Na2S solution was used to precipitate the metal Zn.

Effect of biomass concentration of biosorption[4]

The effect of biomass concentration on the removal of heavy metals, on different biomass concentrations, 2,4,6,8, and 10µl of bacterial biomass, were added to 10ml of metal stock solution taken in 50ml conical flasks. The resulting mixtures were then kept in a room temperature for 24hrs under similar conditions along with their corresponding reference solution.

Effect of pH on biosorption

The effect of pH on the biosorption of metals were examined by adding 10ml of each metal stock solution to 10µl of the bacterial biomass taken in 50ml of conical flasks at different pH such as 5,6,7,8,9 and 10. The resulting mixture was then kept in room temperature for 24hrs. The studies were carried out at room temperature and similar incubation period for all the metals.

Effect of contact time of biosorption

The effect of contact time on the removal of heavy metals were established by 10µl of bacterial biomass suspended in 10ml of each metal solution in 50ml conical flasks in an room temperature at 35°C. The studies were carried out at the corresponding optimal biomass concentration, pH, and temperature in the reaction solution for each metal at predetermined intervals of time.

Effect of temperature on biosorption

The effect of temperature on the removal of heavy metals by bacterial biomass was examined by dissolving sand maintaining the corresponding optimal biomass concentration, pH and optimal time for each metal. The reaction mixtures were kept for 24hrs at different temperatures like 25°C, 30°C, 35°C, 40°C and 45°C to observe the temperature effect.

Statistical analysis

Mean and standard deviation were calculated to facilitate the comparison of the data.

RESULTS AND DISCUSSION

Isolation and identification of microorganisms

In this present study two different bacterial colonies were observed. The isolated colonies were named as IB1 and IB2. Isolated two colonies were identified by morphological and biochemical characteristics and the results are present in TABLE 1. The morphological and biochemical test results were compared with Bergey's Manual of Systematic Bacteriology and the IB1 and IB2 are named as *Pseudomonas aeruginosa* and *Klebiella pneumoniae*.

TABLE 1 : Identification of isolated bacterial colonies

S. No.	Morphological and biochemical characterization	IB1	IB2
1.	Gram staining	Gram -	Gram -
2.	Motility	Motile	Non motile
3.	Shape	Rod	Rod
4.	Indole test	Negative	Negative
5.	Methyl red	Negative	Negative
6.	Voges proskaeur test	Positive	Negative
7.	Citrate utilization test	Positive	Positive
8.	Triple sugar iron agar test	AG	Negative
9.	Starch hydrolysis	Negative	Negative
10.	Urease hydrolysis	Negative	Positive
11.	Oxidase	Negative	Positive
12.	Catalase	Positive	Positive
13.	Carbohydrate	Negative	AG

AG - Acid/Gas

Biomass characterization

The biosorption of Zn, Mg, and Ba by *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* in free and immobilized cells were analysed. The both free cell and immobilized cells of *Pseudomonas aeruginosa* showed maximum sorption of heavy metals (Zn, Mg, and Ba) compared with *Klebsiella pneumoniae*. Both the organism had high effect on Ba (42±0.79%), moderately on Mg (33±0.18%) and less on Zn (18±0.78%). Among this study Barium heavy metal solution has maximum level of sorption by both organisms compared with other heavy metals. The results were presented in TABLE 2 and Figure 1.

TABLE 2 : Biosorption of heavy metals by molybdenium blue method

S.No.	Heavy Metal	% of sorption (Mean±SD)			
		Pseudomonas aeruginosa		*Klebsiella Pneumoniae*	
		Free cell	Immobilized cell	Free cell	Immobilized cell
1.	Zinc	7±0.27	18±0.78	3±0.37	18±0.78
2.	Megnesium	13±0.84	33±0.18	5±0.37	21±0.84
3.	Barium	14±0.84	42±0.78	8±0.50	31±0.86

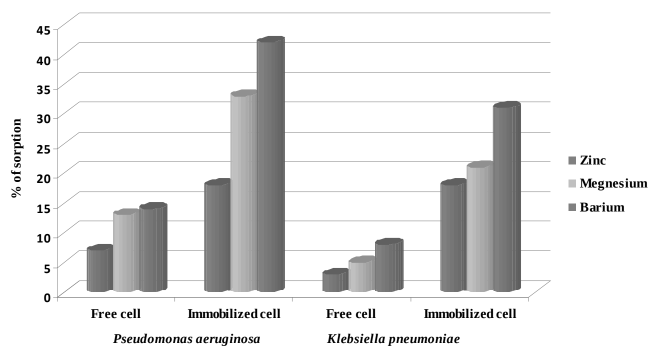

Figure 1 : Biosorption of heavy metal by *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*

In the present study both the free and immobilized cells of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* maximum sorption of heavymetal Zn, Mg and Ba were compared with free cell both the organisms had high effect on Ba and moderately on Mg and less on Zn. Similar results were reported by Lopez *et al.*, 1997; Costa *et al.*, 1991 both the free and immobilized cells of *Halobacterium eutribum* are capable of removing the selected heavy metals from the water with in the stipulated period, sorption is high in immobilized condition.

Gravimetric method

The % of sorption capacity was calculated by Gravimetric methods. The heavy metals sorption ability of *Pseudomonas aeruginosa* was higher than *Klebsiella*

TABLE 3 : Biosorption of heavy metal gravimetric method

S.No.	Heavy Metal	% of sorption (Mean±SD)	
		Pseudomonas aeruginosa	*Klebsiella pneumoniae*
1.	Zinc	7.80±0.97	3.54±0.54
2.	Megnesium	9.16±0.76	2.37±0.63
3.	Barium	10.39±0.78	1.53±0.87

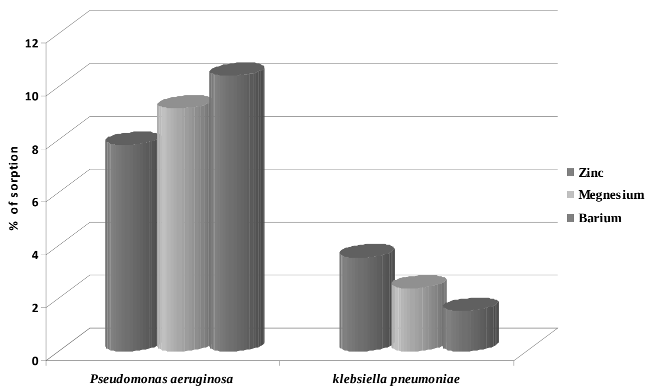

Figure 2 : Biosorption of heavy metal gravimetric method

pneumoniae. The results were presented in TABLE 3 and Figure 2.

In this study % of sorption capability was calculated by using Gravimetric method. *Pseudomonas aeruginosa* has high ability of heavy metal sorption such as Zn, Mg, and Ba compared with *Klebsiella pneumoniae* sorption ability of Zn, Mg and Ba. The present results were compared with Benzawadamanikumar and Suryanarayaraju, 2008. The gravimetric estimation of sorption ability of heavy metal such as Cu, Cd, Ba, Mg, Zn and Pb.

Effect of biomass concentration on biosorption

The reaction mixtures were incubated with varied initial concentration of biomass to verify the change on the sorption capacity of the bacterial biomass. The biosorption concentration was increased simultaneously heavy metal sorption ability also increased when the biomass concentration increased. The results were presented in TABLE 4 and Figure 3 & 4.

Likewise biosorption of metals increase gradually with increase in the concentration of biomass till it get its equilibrium because of increasing adsorption surface area[19].

Effect of pH

The biosorption of heavy metal optimized by different pH value. Both organisms in the alkaline pH 10 the sorption capacity on metals was comparatively higher than acidic and neutral pH. The results were presented in the TABLE 5 and Figure 5 & 6.

TABLE 4 : Effect of initial biomass concentration on sorption of heavy metal

S. No.	Heavy Metal	Biomass concentration (mg/ml)	% of sorption	
			Pseudomonas aeruginosa	*Klebsiella pneumoniae*
1.	Zinc	2	28	24
		4	45	36
		6	63	44
		8	70	52.5
		10	72.5	60.5
		12	73	70
2.	Magnesium	2	37	23
		4	42	34.5
		6	53.5	42.5
		8	60	47
		10	67	49
		12	69	54.5
3.	Barium	2	39	32.5
		4	52	43.5
		6	57.5	47
		8	60.5	50.5
		10	66	63
		12	72.5	69.5

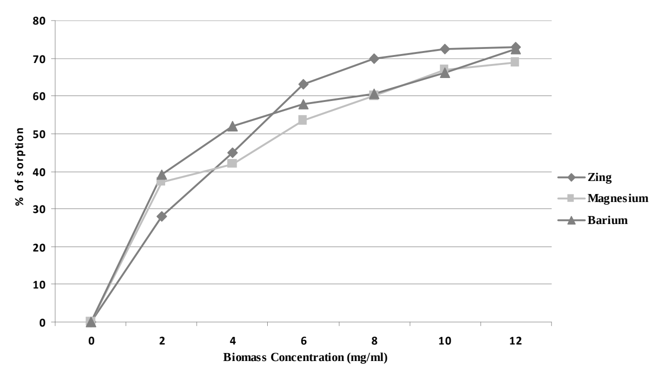

Figure 3 : Effect of biomass concentration on biosorption of heavy metal by *Pseudomonas aeruginosa*

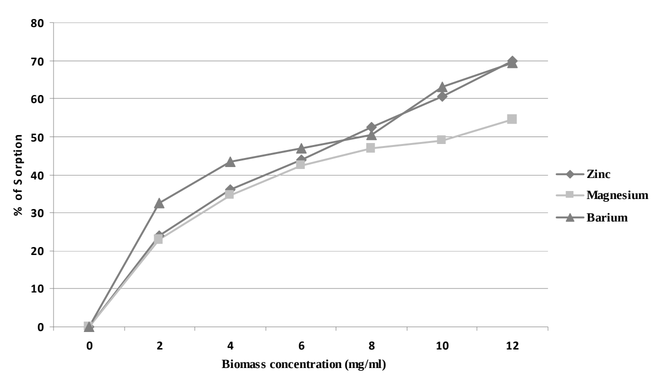

Figure 4 : Effect of biomass concentration on biosorption of heavy metal by by *Klebsiella pneumoniae*

TABLE 5 : Effect of pH on sorption of heavy metals

S. No.	pH	Heavy Metal	% of sorption *Pseudomonas aeruginosa*	% of sorption *Klebsiella Pneumoniae*
		Zinc	7	5.5
1.	5	Magnesium	8	6
		Barium	11	9
		Zinc	9	8.5
2.	6	Magnesium	11.5	10
		Barium	20	17
		Zinc	11	9.5
3.	7	Magnesium	21	17.5
		Barium	34	26
		Zinc	14.5	12
4.	8	Magnesium	23	21
		Barium	42	34.5
		Zinc	18.5	15
5.	9	Magnesium	29	27
		Barium	47.5	42
		Zinc	25	22
6.	10	Magnesium	32	30
		Barium	52.5	47.5

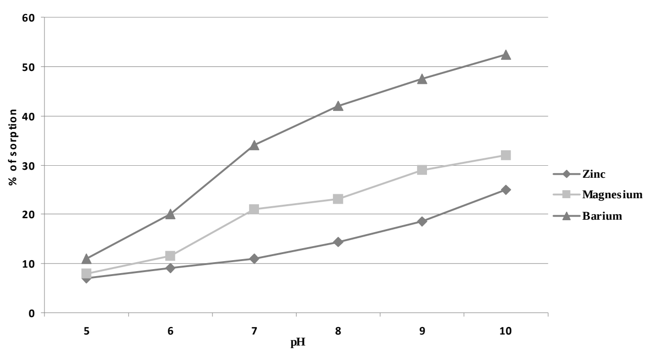

Figure 5 : Effect of pH on sorption of heavy metal by *Pseudomonas aeruginosa*

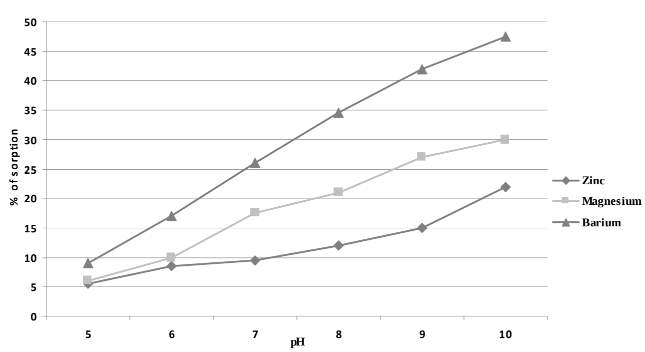

Figure 6 : Effect of pH on sorption of heavy metal by *Klebsiella pneumoniae*

In this study both organisms in the alkaline pH 10 the sorption capacity on metals was comparatively higher than acidic and neutral pH. In the same manner in highly acidic conditions, the sorption was very low for all the metals[6]. In the pH 8, sorption studies were not conducted due to precipitation support that the initial pH of the solution has a vital role in metal sorption. pH is key in most biological process and one of the main variable factor affecting the biosorption process[8,9,12].

Effect of contact time on biosorption

The effect of incubation period on the biosorption of heavy metals was verified. The effect of incubation time on the sorption of metals is presented in TABLE 6 and Figure 7 & 8. Among this study the biosorption

TABLE 6 : Effect of incubation time on biosorption of heavy metal

S. No.	Heavy Metal	Incubation time (min)	% of sorption *Pseudomonas aeruginosa*	% of sorption *Klebsiella pneumoniae*
		30	20	18
		60	34.5	22.5
		90	42	33
1	Zinc	120	48	37.5
		150	56	42.5
		180	64	56
		30	24	20
		60	39	27
		90	45	37
2	Magnesium	120	57	39.5
		150	61	42.5
		180	63	47
		30	15	13
		60	22	17
		90	34	26
3	Barium	120	42	34.5
		150	48	37
		180	54	42

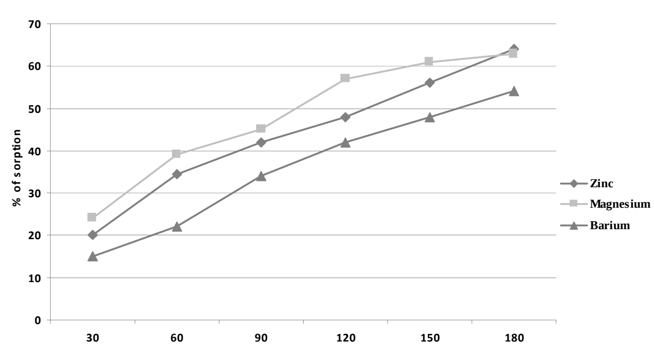

Figure 7 : Optimizing effective time for sorption of heavy metals by *Pseudomonas aeruginosa*

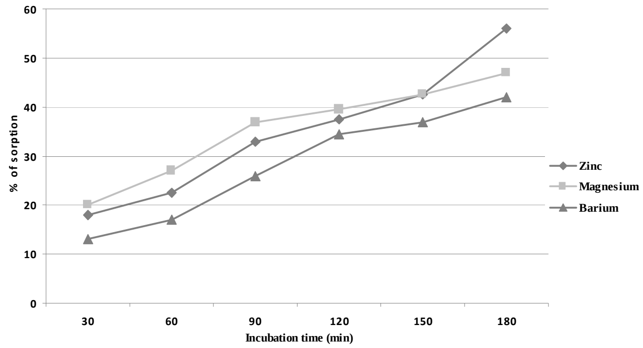

Figure 8 : Optimizing effective time for sorption of heavy metals by *Klebsiella pneumoniae*

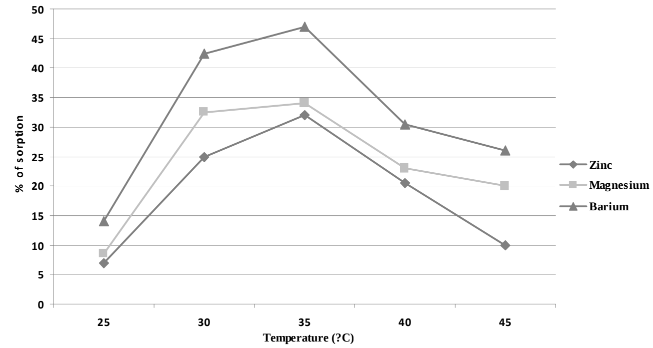

Figure 9 : Effect of temperature on sorption of heavy metal by *Pseudomonas aeruginosa*

ability was maximum in 180min by *Pseudomonas aeruginosa* compared with *Klebsiella pneumoniae*. It is also noted that the sorption ability increase with increase in incubation period. The similar results were obtained that the sorption increase with increase in the incubation period till it reaches the equilibrium at 180 minutes afterwards there is no significant change in the sorption[14].

Effect of temperature

The biosorption of heavy metal optimized by different Temperature. In order to verity the influence of temperature in the sorption process, the studies were conducted at various reaction temperature 25°C, 30°C, 35°C, 40°C and 45°C by maintaining other factors unaltered. The biomass exhibited maximum sorption capability in the temperature range between 30°C-35°C. The results were presented in TABLE 7 and Figure 9 & 10.

TABLE 7 : Effect of temperature on biosorption of heavy metal

S. No.	Temperature	Heavy Metal	% of sorption	
			Pseudomonas aeruginosa	*Klebsiella pneumoniae*
		Zinc	7	4
1.	25?C	Magnesium	8.5	7.5
		Barium	14	10
		Zinc	25	22
2.	30?C	Magnesium	32.5	32.5
		Barium	42.5	39
		Zinc	32	32.5
3.	35?C	Magnesium	34	30
		Barium	47	42.5
		Zinc	20.5	17
4.	40?C	Magnesium	23	19.5
		Barium	30.5	22.5
		Zinc	10	7.5
5.	45?C	Magnesium	20	11.5
		Barium	26	20

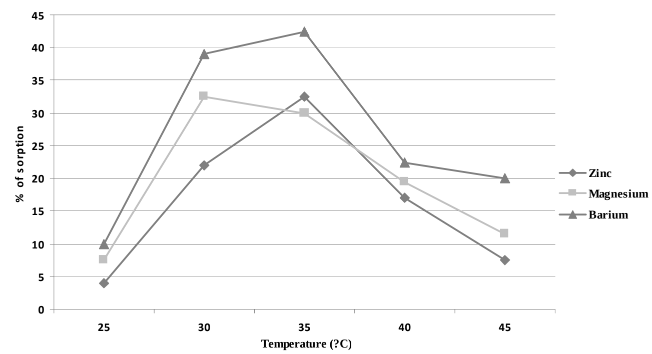

Figure 10 : Effect of temperature on sorption of heavy metal by *Klebsiella pneumoniae*

The similar results were reported that higher temperature could enhance biosorption of heavy metals on non living biomass of Chlorella. So any residual heat in industrial waste water would not suppressed biosorption and it anything might enhance it. But temperature dependence of adsorption is complex mixture[25]. Thermodynamic parameters like heat of adsorption and the energy of activation play on important role in predicting the adsorption behavior and both are shortly temperature dependent[15].

CONCLUSION

Biosorption is accumulation of metal ions on the surface of biomass from dilute aqueous solutions. In this experiment different metals were used such as Zn, Mg, and Ba using free and immobilized cells of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were conducted as function of operating conditions. The different parameters like biomass concentration, pH, contact time and temperature were also analyzed. Hence these organisms are used for the wastewater treatment, which is a cheapest method, due to

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the biosorption capacity of the organisms. The biosorption performance of *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* can be implemented practically for the treatment of wastewater.

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