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

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Abstract

Free and immobilized cells of *Klebsiella pneumoniiae* were studied as biosorbent materials for the removal of Zn, Mg, and Ba from chemical industrial waste water and the influence of pH, incubation time, initial concentration of biomass and temperature were analysed. Compared to free cell, immobilized cells of *Klebsiella pneumoniae* were more efficient in the removal of heavy metals. Immobilized cells of *Klebsiella pneumoniae* showed biosorption of 31 ± 0.86% of Ba at 180 minutes at a temperature of 35ºC and pH 10.

Keywords: biosorption, immobilization, *Klebsiella pneumoniae,* pH, temperature

INTRODUCTION

The gravity of water pollution is of great concern to scientists as the water is the prime necessity of life and extremely needed for the survival of living organisms. The production and utilization of heavy metals increased rapidly since industrial revolution (Inthorn *et al*., 2001). Sometimes, these heavy metals are held in soil as a result of adsorption, chemical reaction and ion exchange (Uzun and Guzel, 2000).Microbial biomass can passively bind large amount of metals, a phenomenon commonly referred to as biosorption (McHale and McHale, 1994). Biosorption is accumulation of metal ions on the surface of biomass from dilute aqueous solution, and it is the property of certain types of inactive, dead or active microbial biomass to bind and concentrate the heavy metals from even very dilute aqueous solutions. The advantage of biosorption lies in both the good performance in metal removal and cost effectiveness. Immobilization of the bacterial cells is one of the important technique by which one can avoid the biomass to separate from liquid environment. (Lopez *et al*., 1997, and Duangrat *et al*., 2005).The metabolic activity of the cell remains constant for longer periods (Lan and Tam, 1998). The mechanism of biosorption is influenced by many experimental factors such as pH, biomass concentration, temperature, etc. The variability of these factors in rear water systems makes it necessary to know as to how they influence the sorption capabilities of biomass (Vegilo and Belochini, 1997).

The present paper deals with the sorption capability and mechanism of free and immobilized cells of *Klebsiella pneumoniae* in the removal of heavy metals and the influence of various environmental factors on the biosorption.

MATERIALS AND METHODS

Isolation of bacteria (Aneja, 2002)

The water sample was collected from chemical industrial waste water at King's chemical industry, Vadaseri, Thiruvarur District, Tamilnadu, India. The organism was isolated by using serial dilution technique. *Pseudomonas* agar medium was prepared by using peptone -20g, Glyceral-10g, Di potassium sulphate-10g, $MgCl_2$-3.5g and the pH 7.4, and inoculated in to Petri plate (Lawrence and Parks 1997). The diluted 0.1ml of sample was spreaded over the medium, and then the plates were incubated at 37ºC. The colonies were observed and identified by Gram staining (Bailey and Scotts, 1996), motility test and biochemical tests (Norris and Ribbon, 1972).

Microorganisms and culture conditions

The biomass of *Klebsiella pneumoniae* was used for the sorption of Zn, Mg, and Ba. The isolates were grown in 250mL Erlenmeyer flasks, each containing 100ml medium (gm/100ml) consisting of Nacl-25g, $MgSO_4$-2g, KCL-0.2g, peptone-0.3g, and sodium citrate-0.5 g at a pH of 7.4. The flasks were inoculated with freshly prepared homogenous culture suspension and incubated at 37°C for one week under constant rotatory conditions (120 rpm). The bacterial mass was harvested by centrifugation at 12000 x g for 10minutes, washed thoroughly with distilled water and dried at room temperature and then used for the biosorption 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 Molybdenum Blue method (Bassett *et al*., 1978).

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METHODS

For biosorption studies, 10mg of dried bacterial biomass was suspended in 10 ml of metal solution contained in 100ml Erlenmeyer flasks, and the flasks were agitated on a rotatory shaker (120 rpm) at 35°C. Samples were withdrawn at definite intervals, centrifuged at 12,000 rpm x g for 5 minutes and the supernatant was analyzed for residual metal content. The residual metal content was determined by using gravimetry method.

Gravimetric estimation

Ten ml of the reaction solution was centrifuged at 12,000 rpm for 10 min. To a 5ml of supernatant solution, a 5ml of 10% precipitating agent was added. The resultant solution was collected, dried in a hot air oven and weighed in an electronic balance (W_f). The same procedure was repeated with the standard metal (1mg/ml) solution to get the initial weight of metal, ($W_{i)}$. The percentage of sorption was calculated as $(W_i - W_f)x\ 100/W_i$. Ten per cent solution of Na_2HPo_4 and $(NH)_4So_4$, 10%Na_2S were used as precipitating agents in the case of Mg, Ba and Zn.

Immobilization of *Klebsiella pneumoniae*

Six per cent sodium alginate solution was prepared by dissolving 6g of sodium alginate in 100ml of distilled water. The resultant solution was autoclaved at 120ºC, 15 lb pressure for 20 min. 100 ml of biomass was properly dissolved in 6% sodium alginate solution and the resultant slurry was taken into a 100ml separating funnel. The slurry was added drop wise to 25% $CaCl_2$ solution to get approximately 2,000 immobilized cell composites.

Effect of Biomass Concentration on Biosorption

The effect of biomass concentration on the removal of heavy metals, was tested by adding different biomass concentrations *viz.*, 2, 4, 6, 8 and 10ml of bacterial biomass to 10ml of metal stock solution taken in 50 ml conical flask. These resulting mixtures were then kept in room temperature for 24 hours under similar conditions along with their corresponding reference solutions.

Effect of pH on biosorption

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

Effect of contact time on biosorption

The effect of contact time on the removal of heavy metals was established by 10ml of bacterial biomass suspended in 10 ml of each metal solution in 50 ml conical flask at 35ºC. The studies were carried out with the corresponding optimal biomass concentration, pH and temperature in reaction solutions with 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 and maintaining the corresponding optimal biomass concentration, pH and optimal time for each metal. The reaction mixtures were kept for 24 hours at different temperature *viz.*, 25ºC, 20ºC, 35ºC, 40ºC and 45°C to observe the effect of temperature .

Statistical analysis: The data were statistically analyzed by the Student's"t" test. The significance level was set at $P<0.05$.

RESULTS AND DISCUSSION

The biosorption of Zn, Mg, and Ba by *Klebsiella pneumoniae* , in free and immobilized condition, was carried out under similar experimental conditions in order to compare the sorption capability of the selected microorganisms for the identified metals. The test organisms were isolated from chemical industrial waste water and identified biochemically.Initially the reaction mixtures were incubated for 24 hours to verify the sorption capacity of *Klebsiella pneumoniae* for different metals. The results are shown in Table 1. From the data it is clear that both the immobilized and free cells of *Klebsiella pneumoniae* were capable of removing the selected metals within the suitable period of time. Sorption was high under immobilized condition. Similar results were reported earlier for both the free and immobilized cells of *Halobacterium eutribum* which were capable of removing the selected heavy metals from

Table 1: Biosorption of heavy metal by *Klebsiella pneumoniae* using Molybdenum Blue and Gravimetric methods

S.No	METHODS	% OF BIOSORPTION (*Klebsiella pneumoniae*)		
		Zn	Mg	Ba
1	**Molybdenium Blue Method:***			
	Free cells	3±0.37	5±0.37	8±0.50
	Immobilized cells	18±0.48	21±0.84	31±0.86
2	**Gravimetric Method:**			
	W_i	564	589	587
	W_f	544	575	578

*Values are represented as Mean± SD

W_i- Initial weight of metal

W_f- Final weight of the metal after drying in hot air oven.

Table 2. Effect of initial biomass concentration, incubation time, pH and temperature on the sorption of heavy metals by *K.pneumoniae*

S. No	Heavy metal	Biomass Concentration (mg/ml)	% of sorption (*Klebsiella pneumoniae*)	Incubation time (mins)	% of sorption (*Klebsiella pneumoniae*)	pH	% of sorption (*Klebsiella pneumoniae*)	Temperature (°C)	% of sorption
1	Zn	0	0	0	0	0	0	0	0
		2	24	30	18	5	7	25	4
		4	36	60	22.5	6	9	30	22
		6	44	90	33	7	11	35	32.5
		8	52.5	120	37.5	8	14.5	40	17
		10	60.5	150	42.5	9	18.5	45	7.5
		12	73	180	56	10	25		
2	Mg	0	0	0	0	0	0	0	0
		2	23	30	20	5	8	25	7.5
		4	34.5	60	27	6	11.5	30	32.5
		6	42.5	90	37	7	21	35	30
		8	47	120	39.5	8	23	40	19.5
		10	49	150	42.5	9	29	45	11.5
		12	54.5	180	47	10	32		
3	Ba	0	0	0	0	0	0	0	0
		2	32.5	30	13	5	11	25	10
		4	43.5	60	17	6	20	30	39
		6	47	90	26	7	34	35	42.5
		8	50.5	120	34.5	8	42	40	22.5
		10	63	150	37	9	47.5	45	20
		12	69.5	180	42	10	52.5		

the water with in the stipulated period with sorption being high in immobilized condition (Lopez *et al*., 1997; Costa and Elite, 1991).

Biomass characterization

The biosorption of Zn, Mg, Ba by free and immobilized cells of *Pseudomonas aeruginosa* was analyzed. Both free and immobilized cells of *Pseudomonas aeruginosa* showed maximum sorption of heavy metals. The immobilized cells had high effect on Ba (31± 0.86%) moderate effect on Mg (21±0.84%) and less on Zinc (18±0.42%). The percentage of sorption capacity was calculated by Gravimetric methods (Table l).

Effect of biomass concentration on biosorption

The reaction mixtures were incubated with varied initial concentrations of biomass to verify the variation in the sorption capacity of bacterial biomass. Heavy metal sorption ability increased when the biomass concentration was increased (Table 2).

Effect of pH

The biosorption of heavy metals was comparatively higher in the alkaline pH 10 to acidic and neutral pH. The results are represented in the Table 2. The role of pH on metal uptake is highly related to the functional groups in the biomass and also the metals chemistry in the solution (Vinojkumar and Kaladharan, 2006). The results obtained in the present study confirm the significant role of pH in the metal sorption process.

Effect of contact time on biosorption

The effect of incubation period on the biosorption of heavy metals was studied (Table 2). The biosorption ability of *Klebsiella pneumoniae* was maximum in 180 minutes. Further it was noted that the sorption increased with increase in the incubation period as it reached the equilibrium in 180 minutes, and after wards there was no significant change in the sorption. The results were similar to Matheickal *et al*., (1999).

Effect of temperature

The biosorption of heavy metals showed that maximum sorption capability was in the temperature range of 30ºC – 35ºC (Table 2).

CONCLUSION

It is concluded that the entrapment of all the metals was higher in the immobilized cell than in free cell. The experiment showed that the biosorption ability of *Klebsiella pneumoniae* could be applied for waste water treatment.

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