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LEAD REMOVAL BY FREE AND IMMOBILIZED CELLS OF *KLUYVEROMYCES MARXIANUS*

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ABSTRACT

The ability of the living biomass of the yeast Kluyveromyces marxianus to remove lead from the aqueous solution has been examined in batch mode. The biomass yield is found to be maximum with the YPL medium. The optimum pH for lead removal by the yeast is found to be 5. Above 300mg/L of initial lead concentration, minimal microbial growth and mere lead removal was observed. The maximum % removal of the yeast is 99.12 at 20mg/L initial lead concentration. Increasing the initial metal concentration leads to decrease in the protein content of the yeast. Monod curve is plotted for the growth of the yeast in the presence of the metal. Hanes method gave a better regression fit for the data set with the half saturation constant (Ks) as 37.99mg/L, and the maximum specific growth rate (μmax) as 0.28/h. Immobilization of the yeast is carried out. The density and diameter of a single immobilized bead is determined as 4.8*10-3g/ml and 0.30cm, respectively. The viable cells immobilized in a single bead are 2*109CFU/ml. Batch study is conducted for the removal of lead ions with the immobilized yeast. The optimum number of beads is being fixed as 200 with a removal of lead as 94.02% for 100ppm of initial lead concentration. The experimental equilibrium data fits both models well, thus illustrating the fact that the use of Ca-alginate as an entrapment matrix for biosorption of lead could be modeled using both the Langmuir and Freundlich isotherms. Pseudo-first order model fits well for the pre-equilibrium data. Column studies for the immobilized yeast were conducted. The immobilized beads were able to remove lead ions completely from a solution of with the initial metal concentration of 25ppm (1500ml) with a flow rate of 10ml/min and 25cm bed height.

1. Introduction

Various human activities like ore mining and industrial processes disrupted the natural biogeochemical cycles, causing increased depositions of heavy metals in the environment [1]. Many industries especially metal industries, electroplating, battery manufacturing, pigment and dye industries, lead smelting and internal combustion engine fueled with leaded petroleum etc. discharge lead as a waste to the environment [2]. Lead has a detrimental effect on the environment where it accumulates throughout the food chain [3].

Conventional methods for removing metal ions from aqueous solution have been suggested, such as chemical precipitation, filtration, ion exchange, electrochemical treatment, membrane technologies, adsorption on activated carbon, evaporation etc [4,5]. However, these methods are ineffective, especially when metal ion concentration in aqueous solution is among 1 to100 mg/L, also produce large quantity of sludge and are extremely expensive [6]. In this endeavor, microbial biomass has emerged as an option for developing economic and eco-friendly waste water treatment process [7]. A wide variety of fungi, algae and bacteria are now under study or are already in use as biosorbents for heavy metal remediation [8-10].

Uptake of heavy metals by microbial cells is a result of the mechanisms of bioaccumulation and biosorption. The term biosorption, some times referred to as physical adsorption, describes the ability of inactive, dead or living biomass to bind to heavy metals or contaminants present in dilute solutions. The cell-wall structure is mainly responsible for this property. The term bioaccumulation refers to the metabolically driven uptake by active living cells [11, 12].

Yeasts are an inexpensive, readily available, easily cultivated using simple fermentation techniques and inexpensive growth media. Furthermore, yeast cells retain their ability to accumulate a broad range of heavy metals to varying degrees under a wide range of external conditions [13]. The yeast has been studied by many investigators as a biosorbent for removal of heavy metals [3, 9, 11, and 14].

K. marxianus is yeast which is inexpensive and readily available source of biomass for heavy metal removal from wastewater. Investigations conducted by several researchers demonstrated that K. marxianus is capable of accumulating heavy metals such as Cu (II) and U (VI) [6, 12, 13, 15, and 16].

The use of freely suspended biomass may be plagued with operational difficulties, but immobilized microbial cell systems could provide additional advantages: ease of regeneration and reuse of the biomass, easier solid liquid separation and minimal clogging in continuous flow systems [18]. Materials that have been successfully used for cell entrapment include agar, agarose, alginate, k-carrageenan, polyacrylamide, polyurethane, cellulose, collagen, chitin, chitosan, polysulfone and epoxy resins [19]. Ca-alginate has been one of the most extensively investigated biopolymers for binding heavy metals from dilute aqueous solutions [18, 20-22].

Information is available on the use of immobilized Kluyveromyces marxianus biomass for other applications [23, 24], but no study has been conducted on the use of the immobilized biomass for removal of heavy metal ions. Therefore there is a need to study the performance of the immobilized yeast system with the other heavy metal ions. In this study, freely suspended and immobilized cells of Kluyveromyces marxianus were chosen for equilibrium and kinetic studies for biosortion of lead in batch system. A column study with the immobilized yeast is conducted in order to perform continuous operations.

2. Materials and Methods

2.1 Microorganism and Growth Conditions

The Kluyveromyces marxianus (MTCC Code 95) used in this study was obtained from MTCC (Microbial Type Culture and Gene Bank), Institute of Microbial Technology, Chandigarh, India. The composition of the culture medium used was 1% Dextrose, 0.5% peptone, 0.3% yeast extract and 0.3% malt extract.

2.2 Metal solution

All chemicals and reagents used for experiments and analyses were of analytical grade. Stock solutions of 1000 mg/L Pb (II) was prepared from Pb (NO3)2 in distilled water. The solutions were diluted as required to obtain working solutions. Samples were determined using Varian model Spectraa 220 Atomic Absorption spectrophotometer.

2.3Media optimization study

Various media were selected for the media optimization study. The media used were Sabouraud [25], YD [26], YP [27], YPL [28], Maltose [28], YM [29] and Mineral [30]. Batch study was conducted with the entire above mentioned medium. Growth yield was characterized by performing a viable cell count. Colony-forming-units (CFU) were measured by serial plating technique on yeast-malt extract agar.

2.4 Growth Curve Study

Growth curve of the yeast was determined in the presence and absence of the metal. Inoculum concentration of 1% (v/v) was used for the study. The cells were grown at 30ºC in the growth medium broth with metal (20mg/L) and without metal. Samples were collected at various time intervals and the OD of the culture was determined at 600nm, spectrophotometrically.

2.5 MIC

Heavy metal resistant yeast was determined by plate diffusion method [8]. The metal solution was used in varying concentrations ranging from 100 to 400ppm. Stock solution of the metal salt was prepared in sterile distilled water and was added to the agar medium (pH-7.0) in various concentrations which were then inoculated with yeast. The plates were incubated at 25ºC for 48h. The lowest concentration of the metal, which inhibits the growth of the microorganism, was considered as the MIC of the metal against the yeast.

2.6 Batch study

Batch adsorption experiments were carried out in growth medium by shaking the flasks at 150rpm using rotary shaker for 48h. The effect of various parameters on growth and removal of lead ions was studied. Samples were collected at regular time intervals (2h), centrifuged and the concentration of metals in the supernatant solution was analzed. The effect of pH was studied for a range of 3 to 7 with a growth medium with initial metal concentration of 20mg/L Pb (II). The temperature study was conducted by incubating the growth medium with initial metal concentration of 20mg/L Pb (II) was prepared and incubated at varying temperatures such as 15 to 45oC at optimum pH and with optimum amount of inoculum concentration. The effect of initial metal concentration was studied by preparing growth medium with a solution of 20-500 mg/L Pb (II) was prepared and maintained at optimum conditions.

2.7 The estimation of total protein content of the yeast

Growth medium with a solution of 20-500 mg/L Pb (II) was prepared and batch study was conducted. At the stationary phase of the organism, 10ml was withdrawn and centrifuged at 10,000rpm for 5minutes. The supernatant was discarded and the biomass was dried. Dried samples of washed cells were incubated in 5ml of 1N NaOH at 90oC for 10minutes to solubilize cellular protein. The cellular protein was estimated using Lowry method [31].

2.8 Monod Model

Growth medium with a solution of 20-500mg/L Pb (II) was prepared and its pH was adjusted to optimum value. The medium was then inoculated with the optimum amount of inoculum and incubated at optimum temperature. The growth kinetics of the organism was determined and the Monod curve was plotted.
The relationship of specific growth rate to substrate concentration often assumes the form of saturation kinetics. In this case we assumed initial lead concentration (Substrate), as growth rate limiting substrate for the microorganism. The Monod model is stated as:

$$\mu = \frac{\mu_{max} * S}{K_s + S} \qquad \text{..... (1)}$$

Where, μ = Specific growth rate, time-1, μmax = Maximum specific growth rate when S>>Ks, S = Substrate concentration, mg/L, Ks = Saturation constant or half-velocity constant, mg/L.

2.9 Lineweaver-Burk Plot

The Lineweaver-Burk equation is obtained by taking the inverse of the Monod Model.

$$\frac{1}{\mu} = \left(\frac{K_s}{\mu}\right) * \left(\frac{1}{S}\right) + \left(\frac{1}{\mu_{max}}\right) \qquad \text{..... (2)}$$

The slope is Ks/μmax and the intercept is 1/μmax from plotting 1/μ versus 1/S. The Lineweaver-Burk method has been widely used to determine the kinetic parameter values.

2.10 Hanes Plot

The Hanes plot is obtained by multiplying the Lineweaver-Burk equation by the substrate concentration.

$$\left(\frac{S}{\mu}\right) = \left(\frac{K_s}{\mu_{max}}\right) + \left(\frac{1}{\mu_{max}}\right) * S \qquad \text{..... (3)}$$

The slope is 1/μmax and the intercept is Ks/μmax of the plot S/μ versus S. This method is the most recommended for many different situations, because it minimizes the distortions in experimental error.

2.11 Immobilization of the yeast

In the stationary phase of growth, yeast cells were centrifuged and resuspended in 3% sodium alginate. The ratio of Na-alginate to biomass was 100:3. This mixture was dropped into 0.5M calcium chloride solution. The drops of Na-alginate solution gelled into spheres upon contact with calcium chloride solution. Ca-alginate immobilized yeast particles were stored in calcium chloride solution at 4°C for 24h to complete gel formation. In this way insoluble and stable immobilized beads were obtained [32, 33].

2.12 Batch study for immobilized yeast

Batch experiments were carried out by shaking the flasks at 150rpm using a rotary shaker. The effect of various parameters on the metal removal capacity of the immobilized yeast was studied. Samples were collected at regular intervals and the concentration of metal was analyzed. Effect of immobilized bead number on lead removal was studied by inoculating the 100ml of lead solution (100ppm) with immobilized beads ranging from 50-300 beads and incubated at optimized conditions (35°C, pH 5.0). The pH was adjusted to 5.0 and incubated at 35°C in rotary shaker at 150rpm. The effect of initial metal concentration on the lead removal was studied by inoculating optimum number of beads in 100ml of lead solutions at various concentrations ranging from 50-300ppm. It was then incubated under the optimized conditions (35°C, pH 5.0).

2.13 Modeling of batch biosorption – Isotherm and kinetics studies

Biosorption isotherms are expressed in terms of relationship between the concentration of bioadsorbate in the liquid and the amount of adsorbate adsorbed by the unit mass of adsorbent at a constant temperature. Langmuir [22], Freundlich [23], isotherm models which are available in the literature were used to describe the equilibrium data. In the present study, the kinetics for metal ions removal by immobilized yeast has been verified by pseudo first [26] and second [27, 28] order kinetics to understand the behavior of the immobilized yeast.

2.14 Column studies

Continuous flow experiments were conducted in a glass column (Inner diameter=2cm, Total column height=35cm). At the bottom of the column, sterilized non-absorbant cotton was placed followed by 1cm high layer of glass beads. The column was then packed densely with immobilized beads and operated in an up flow mode at room temperature. The flow rate was regulated with peristaltic pump. The effect of flow rate was carried out at the bed height of 5cm at various flow rates of 10, 15 and 20ml/min with the initial metal concentration of 50ppm. The effect of initial metal concentration with the inlet metal concentration of 25, 50, 75 and 100ppm was studied (bed height= 5cm and at optimum flow rate). The lead removal capacities by the immobilized beads at various bed heights (5, 15 and 25cm) were studied (initial inlet metal concentration of 25ppm and at optimum flow rate). Samples were taken from the effluent at timed intervals and analyzed for lead ions. The experiment was continued until a constant concentration of lead ion was obtained.

3. Results and discussion

3.1 Media optimization

The cell density in terms of CFU/ml for the various media studied is presented in Table 1. The lactose medium produced a maximum yield of 4×1010CFU/ml. The YPL medium is chosen for further study. The optimization of the concentration of lactose, yeast extract and peptone is determined. Table 2 describes medium used for study and its corresponding viable cell counts. The concentration of lactose is varied from 0.5-6%. The maximum growth yield is obtained in the medium with the lactose concentration of 3% with a cell density of 6×1010CFU/ml. The optimum concentration of yeast extract and peptone are found to be 2% and 2.5%, respectively. The cell density of their corresponding media is 7×1010CFU/ml and 9×1010CFU/ml.

Table 1: Media optimization

S.no	Medium	Cell density (CFU/ml)
1	Sabouraud	2×10^{10}
2	YD	3×10^{10}
3	YP	3×10^{10}
4	YPL	4×10^{10}
5	Maltose	4×10^{9}
6	YM	3×10^{9}
7	Mineral	1×10^{9}

Table 2: Optimization of concentration of yeast extract peptone lactose media

S.no	Medium			Cell density (CFU/ml)
	Lactose (g)	Yeast extract (g)	Peptone (g)	
1	0.5	1	1	1×10^{9}
2	1	1	1	2×10^{9}
3	2	1	1	5×10^{10}
4	3	1	1	6×10^{10}
5	4	1	1	5×10^{10}
6	5	1	1	3×10^{10}
7	6	1	1	2×10^{10}
8	3	0.25	1	1×10^{9}
9	3	0.5	1	4×10^{9}
10	3	1	1	2×10^{10}
11	3	1.5	1	3×10^{10}
12	3	2	1	7×10^{10}
13	3	2.5	1	2×10^{10}
14	3	3	1	1×10^{10}
15	3	2	0.25	1×10^{9}
16	3	2	0.5	2×10^{9}
17	3	2	1	4×10^{10}
18	3	2	1.5	2×10^{10}
19	3	2	2	1×10^{10}
20	3	2	2.5	9×10^{10}
21	3	2	3	1×10^{10}

3.2 Growth curve

The growth curve of the yeast in YPL medium with metal concentration of 20mg/L and without metal in the medium is shown in the figure 1. Both the growth curves followed similar pattern. The yeast cells exhibited an initial lag phase upto 18h. The exponential phase extended from 18h to 24h. Stationary phase extended till 70h. The yeast grown in medium with metal exhibited higher growth levels than in medium without metal. This may be due to the reason that lead in minimal quantities (20mg/L) enhance the growth of the yeast cells.

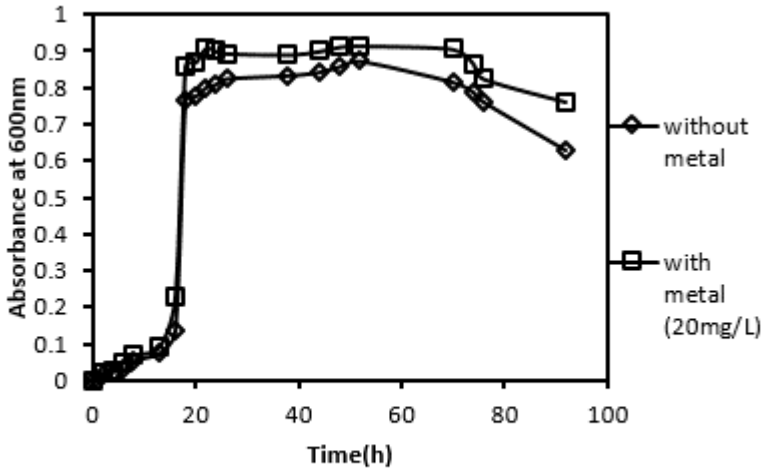


Fig 1: Growth curve for the organism

The growth curve of Kluyveromyces marxianus in YM medium at 30^{o}C exhibited an initial lag phase upto 13h and the exponential phase extended from 13 to 24h. Under the same experimental conditions Saccharomyces cerevisiae exihibited an initial lag phase upto 6h and the exponential phase extended from 6 to 15h [16].

3.3 MIC

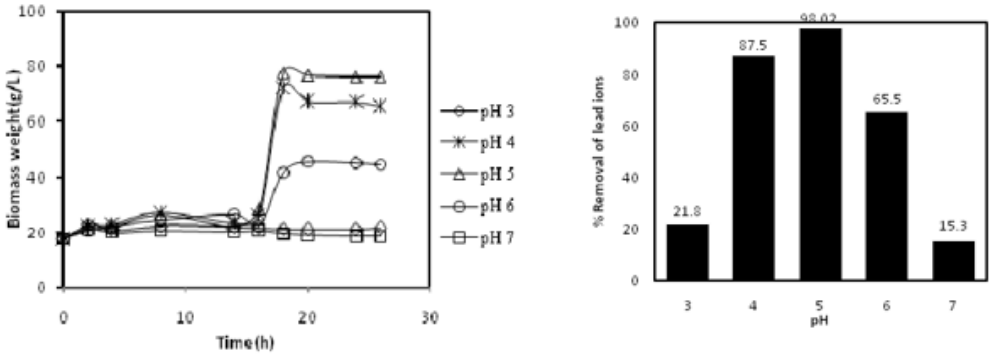
The yeast cells were able to grow in the growth medium containing the metal concentration of up to 300ppm. No change in its characteristics was observed. Above 300ppm no growth was observed in the medium agar.

3.4 Batch study

3.4.1 Effect of pH

Effect of pH has been reported as a key parameter in most biological processes and controls the growth and/or the adsorption capacity of substances. In addition, solution pH influences the adsorption of metals differently. The effect of pH on the growth of the yeast species is shown in the figure 2(a). Results of sorption efficiency by the yeast species are presented in the figure 2(b). The percentage uptake of lead ion by the yeast species ranged from 15.3-98.02%. Metal uptake by the living biomass was low initially at pH 3 before rising to a maximum value and then dropped slightly at pH 6 and decreased rapidly at pH 7. The highest sorption of 98.02% at pH 5 was obtained for this yeast species. The low removal capacity at pH values below 5 is attributed to the competition of hydrogen ion with metal ion on the sorption site. Thus at low pH, due to the protonation of binding site resulting from high concentration of proton, a negative charge intensity on the site is reduced which results in the reduction or inhibition for the binding of metal ion. Most of the microbial surfaces are negatively charged due to the ionization of functional group thereby contributing to metal binding. The increase in pH from 3 to 5 is due to the strong relations of bioaccumulation to the number of surface negative charge, which depends on the dissociation of functional group.

With further increase in pH, the percent removal of metal decreased. With increase in pH beyond 6 the lead removal rate decreased, which might be due to the osmotic changes and hydrolyzing effect. Substantial precipitation of lead as lead hydroxide occurs at high pH values. The formation of hydroxide precipitate reduces the amount of free lead ions, which accumulates to the organism [9]. Similar results were obtained for lead removal at pH 5 by waste brewery yeast [14]. Maximum copper (II) uptake (6.44mg/g dry weight) was observed at pH 5.0 for K. marxianus [6].



(a)Variation of yeast growth with pH

(b)Effect of pH on removal of lead

Fig 2(a) and (b) The effect of pH on the growth of yeast (initial metal concentration of lead =20ppm, temperature =35^{0}C)

3.4.2 Effect of Temperature

The effect of temperature on the growth of the yeast at different temperatures was optimum growth at 35oC. When the growth temperature of a microorganism is reduced, the initial lag phase extends, the growth rate decreases and the final cell number usually decreases. At low temperatures, the organism produced longer lag phase extending upto 40h. The percentage removal of lead ions at different temperatures is shown in the figure 3. The percentage removal of lead ions is found to be high at 35^{o}C, i.e. 99.11%. An optimum temperature of 30oC for uranium uptake by *Kluyveromyces marxianus* was observed [15].

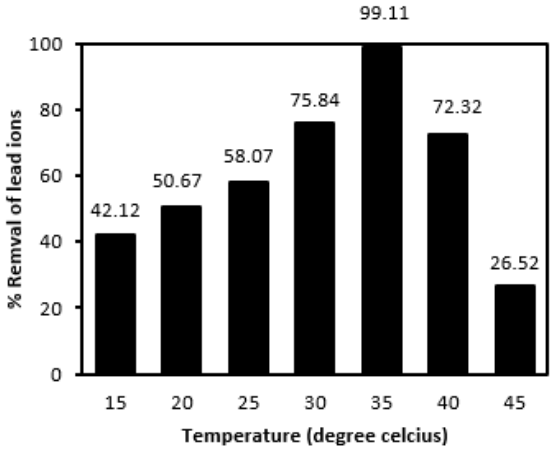


Fig 3: Effect of temperature on removal of lead (Initial concentration of lead= 20ppm, pH=5)

3.4.3 Effect of Initial Metal Concentration

The effect of initial lead concentration on the growth of the yeast is shown in the figure 4 (a) & (b). The yeast cells were able to grow upto 300mg/L of lead. The optimum initial metal concentration is found to be 200mg/L. *Kluvernomyces marxianus* growth was sensitive to high concentrations of lead with an extension in lag phase duration, correlated a decrease in yeast production.

The % removal of lead ions varied from 99.12%-87.94%. Over 300mg/L of initial lead concentration, minimal microbial growth and mere lead removal was observed. The maximum % removal of the yeast is 99.12 at 20mg/L initial lead concentration. The figure 5 shows that the lower initial lead concentrations favored higher % removal.

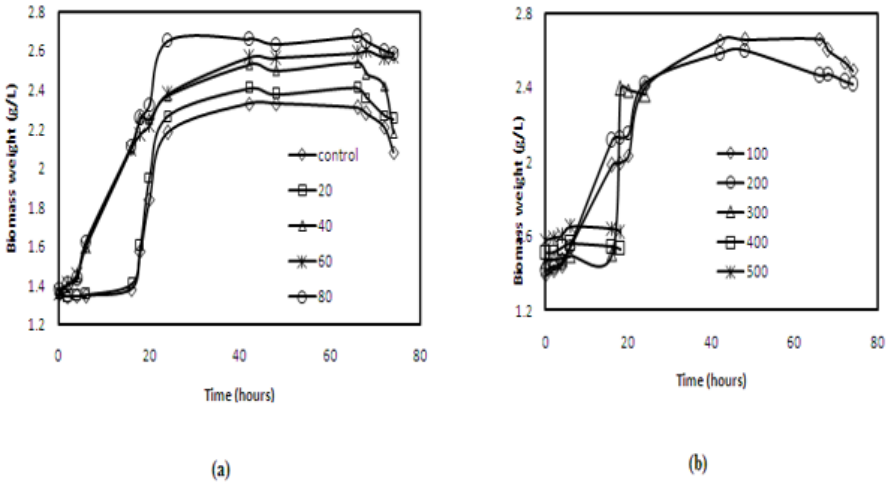


Fig 4(a) and (b) Variation of yeast growth with different metal concentration

(pH=5, Temperature=35^{0}C)

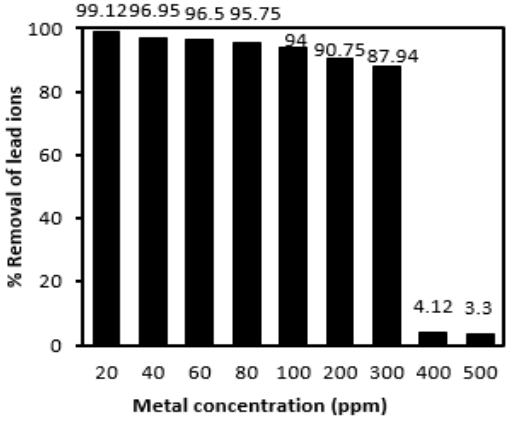


Fig 5: Effect of initial metal concentration on removal of lead (Temperature=35°C, pH=5)

The copper (II) uptake capacity of K. marxianus for 200mg/L was 123.8mg/g. *Kluvernomyces marxianus* growth was sensitive to high concentrations of copper (II) [6]. The residual biomass from the yeast strain *Kluyveromyces marxianus* IMB3 had an observed maximum biosorption capacity of 120mg/g dry weight of biomass [16]. The biosorption of cadmium and lead ions from artificial aqueous solutions using waste baker's yeast biomass was investigated and the highest metal uptake of 17.49mg/g for Pb was obtained by ethanol treated yeast cells [9]. The maximum uptake capacity of the baker's yeast was 20.4mg/g dry biomass when using 150mg/L initial lead (II) concentration [11].

3.4.4 Effect of initial metal concentration on total protein

The effect of lead ion concentration on the total protein content of the yeast is shown in the figure 6. The total protein of the yeast is found to be 7.54mg/L under normal conditions in the absence of lead. Increasing the initial metal concentration leads to decrease in the protein content of the yeast. This is mainly due to the binding of the yeast cells to the lead ions. Metals such as Hg, Cu, Cr variable effects on protein biosynthesis, at respective concentrations of 0.2, 0.5, 0.5 and 1mM, did not affect protein synthesis in B. thuringiensis but, on the other hand, Co at 0.1mM metabolism showed a significant inhibitory effect on protein biosynthesis [30].

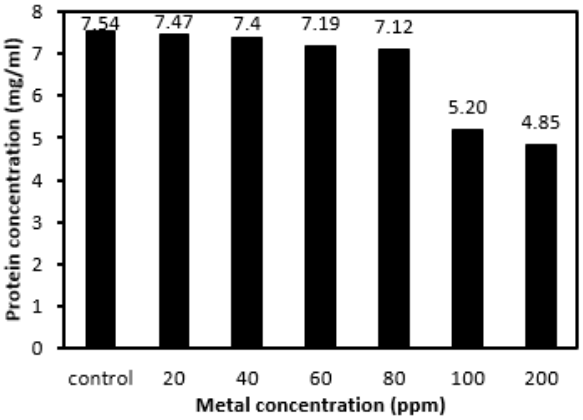


Fig 6: Effect of initial metal concentration on total protein (Temperature=35°C, pH=5)

3.4.5 Monod curve

The cell growth increased with increase in initial concentration of lead. The specific growth rate as a function of initial substrate concentration is presented in figure 7. It was inferred that til initial substrate concentration of 80mg/L, the specific growth rate increases. After 80mg/L of substrate concentration, the specific growth rate is found to be stationary upto 200mg/L of lead.
The growth rate of *Kluvernomyces marxianus* was found to be 0.260/h with the initial copper concentration being 49.9mg/L [6]. Maximum copper (II) uptake (8.0mg/g dry weight) and specific growth rate of *Kluvernomyces marxianus* (0.075/h) was observed at pH 4.0 [13].

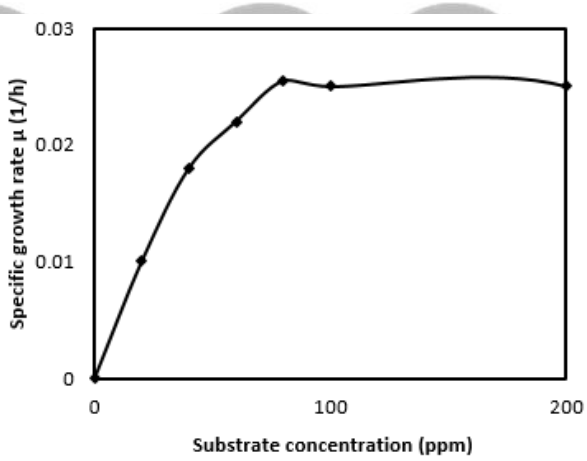


Fig 7: Specific growth rate as a function of metal concentration

3.4.6 Lineweaver-Burk Plot and Hanes Plot

From the regression equations we are able to determine the growth kinetic parameters (Ks and the μmax). The half saturation constant (Ks) is 37.99mg/L, and the maximum specific growth rate (μmax) was 0.28/h for the Hanes method, and the half saturation constant (Ks) is 39.907mg/L, and the maximum specific growth rate (μmax) was 0.028/h for the Lineweaver-Burk method. The maximum specific growth rates and the half saturation constants for both methods gave similar results as indicated in table 3. The Hanes method gave a much better regression fit for the data set of an R2 = 0.990.
The plots of 1/m vs 1/S obtained in the presence of increasing copper (II) concentrations for Kluvernomyces marxianus showed that the inhibition obeyed the non-competitive inhibition model. The average K value was determined as 394.7922mg/L [13]. The maximum growth rate and maximum biomass yields were observed in the fermentation carried out without any lead (II) additions. Growth was affected at the lowest lead (II) (10mg/L) addition. The maximum specific growth rate (μmax) was found to be 0.269/h for 3mg/L of initial lead concentration [11].

3.5Batch study for immobilized yeast

3.5.1 Effect of bead number

The effect of bead number on the percentage removal of lead by the yeast is shown in the figure 8(a).The initial metal concentration was maintained at 100ppm. The percentage removal of lead ions is found to increase with increase in the number of beads. The percentage removal of lead ions does not increase significantly though there is an increase in bead number from 200 to 300. Hence, the optimum number of beads is being fixed as 200 with a removal of lead as 94.02%.

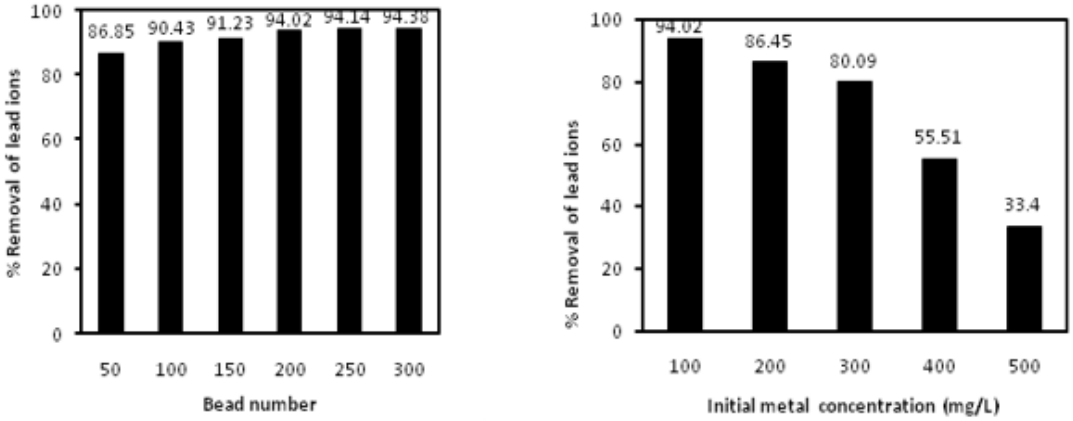


Fig 8: Effect of bead number and initial metal concentration on removal of lead

a= (Temperature=35°C, pH=5)

b= (Temperature=35°C, pH=5, bead number=200)

3.5.2Effect of initial metal concentration

The effect of initial lead concentration on the % removal of lead ions by the immobilized yeast is shown in the figure 8(b). As initial lead concentration increases, the % removal decreases. The % removal of lead ions varied from 33.4-94.02%.

A removal efficiency of 90% for the metal lead was obtained by treating real waste water by immobilized cells of *Aspergillus niger* [33]. The biosorption capacities of Pb and Zn onto the Ca-alginate and both live and inactive immobilized Phanerochaete chrysosporium as a function of the initial concentration of heavy metals ions within the aqueous phase were studied. The saturation values are around 500mg/L for Pb and 300mg/L for Zn [20].

3.5.3Adsorption isotherm

In order to determine if the yeast entrapped systems could be modeled using adsorption isotherms, the two most commonly used adsorption isotherms for biosorption of lead were investigated. The Langmuir constants (Qmax and b) along with correlation coefficients have been calculated from the plot and the results are presented in Table 4. The maximum capacity determined from the Langmuir isotherm defines the total capacity of the beads for Pb (II) ions.

The magnitudes of Kf and n (Freundlich constants) show easy separation of metal ions from aqueous medium and indicate favorable adsorption. The intercept Kf value is an indication of the adsorption capacity of the adsorbent; the slope 1/n indicates the effect of concentration on the adsorption capacity and represents adsorption intensity. The magnitude of Kf and n showed easy uptake of Pb(II) from aqueous medium with a high adsorption capacity of the entrapped live yeast. As seen from Table 4, n value was found high enough for separation. The experimental equilibrium data fits both models well (as seen in Table 4), thus illustrating the fact that the use of immobilized beads for biosorption of lead could be modeled using both the Langmuir and Freundlich isotherms.

The ability of residual biomass from the yeast strain Kluyveromyces marxianus IMB3 to function as a biosorbent for uranium has been examined. The calculated value for the biosorption maximum, obtained by fitting the data to the Langmuir model was found to be 130mg/g dry weight biomass [17]. The uranium sorption onto Kluyveromyces marxianus could be better described by a Freundlich isotherm [12]. The basidio spores of Phanerochaete chryosporium were immobilized in alginate gel beads. The experimental biosorption equilibrium data for Pb and Zn ions were in good agreement with those calculated by Langmuir model [20].

Table 4: Isotherm fit values and correlation constants

Isotherm	Q_{max}(mg/g)	b	n	K	R^2
Langmuir	62.5	0.0019	-	-	0.895
Freundlich	-	-	6.25	1.17	0.476

3.5.4 Kinetic models

In order to analyze the biosorption kinetics of lead ions onto the immobilized cells of beads, the pseudo-first order and pseudo-second order models were tested using experimental data. The corresponding parameters were determined by linear regression and are listed in Table 5. Result obtained indicates that the pseudo-first order model best described the data for biosorption of lead ions onto immobilized beads followed by pseudo-second order model.

The correlation coefficients (R2) for the linear plots using the pseudo-first order model ranged between 0.983 and 0.996 while the correlation coefficients for the linear plots for the pseudo-second order model ranged between 0.968 and 0.986. This result suggested that the pseudo-second order model is less suitable to describe this biosorption process and the pseudo-first order model can be adapted to describe this process as it fit accurately with the experimental data. The adequacy of the pseudo-first order model is in agreement suggested that metal uptake by the immobilized beads is controlled by diffusion, because the pseudo-first order model is mathematically equivalent to a mass action rate equation for sorption kinetics seen as a transfer process. By contrast, the pseudo-second order model assumes that the rate limiting step is chemisorption of metal ions onto sorbent binding sites. The interaction between metal ions and binding sites in beads must be a fast step in the sorption process, so that it is not rate-limiting.

Biosorption of uranium (VI) ions by immobilized *Aspergillus fumigatus* beads was investigated in a batch System. The dynamic adsorption model conformed to pseudo-second order model [17]. The sorption of Cr (VI) on immobilized *Trichodrema viride* biomass follows pseudo-first order model [34].

Table 5: Kinetic models for uptake of Pb(II) in batch mode by the immobilized beads

Initial concentration of lead(ppm)	Pseudo first order			Experimental value q (mg/g)	Pseudo second order		
	q_e(mg/g)	K_1 (min^{-1})	R^2		q_e(mg/g)	K_2 (min^{-1})	R^2
100	1.412	0.409	0.991	1.382	2.092	0.002	0.986
200	1.592	0.261	0.983	1.604	2.525	0.00158	0.97
300	1.595	0.236	0.996	1.595	2.77	0.0008	0.968

3.6Column studies

3.6.1Effect of flow rate

The break through curve for the effect of flow rate is shown in the figure 9(a). As flow rate increases, the removal of lead ions decreases. This could be attributed to metal solution that left the column before the attainment of equilibrium with increased flow rate. Thus increase in the residence time of the solution increases the removal percentage. Maximum removal takes place with a flow rate of 10ml/min. 100% removal was observed for a time period of 1h with the flow rate of 10ml/min. The immobilized yeast was able to remove lead ions completely for 600ml of the influent solution.

The effect of flow rate on copper adsorption on to Ca-alginate immobilized C. vulgaris beads was studied. A maximum removal percentage was obtained at a flow rate of 0.8ml/min. The percentage removal of copper with a flow rate of 0.8ml/min was found to be 65.7 [21]. The adsorption of copper ions to Ca-alginate and immobilized Z. ramigera in the packed column was investigated as a function of flow rate. Maximum percentage removal of 94.3 was observed for the flow rate of 3.6ml/min [19]. The performance of Ca-alginate immobilized T. harzianum was investigated as a function of flow rate. The uptake capacity of the immobilized beads at a flow

rate of 2.5ml/min was found to be maximum [18].

3.6.2 Effect of initial metal concentration

As generally expected, a change in the inlet ionic concentration of the feed affected the operating characteristics of the packed column. The break through curve for initial metal concentration was studied and shown in the figure 9(b). The optimum initial metal concentration was found to be 25ppm. As the initial metal concentration increases, the removal of lead ions by the immobilized yeast decreases. With the initial metal concentration of 25ppm, 100% removal was achieved til 900minutes thereby treating 900ml of the influent solution.

Total removal percentage of copper at different inlet metal ion concentrations at the end of 480minutes for the copper adsorption on Ca-alginate immobilized C. vulgaris beads was found to be 83.8% for 50ppm of copper [21]. The adsorption of Cu(II) ions to Ca-alginate and immobilized Z. ramigera in the packed column was investigated. Total Copper removal percentage of the inlet metal ion concentration (49.8mg/L) at the end of 24h was found to be 93.3 [19].Fixed bed break through curves at 25.5, 50 and 100mg/L feed concentration of uranium was obtained for uranium biosorption by Ca-alginate immobilized T. harzianum biomass. The immobilized material could purify 24, 8.5 and 0.5L of 25.5, 50 and 100mg/L uranium solutions respectively before break through occurs [18].

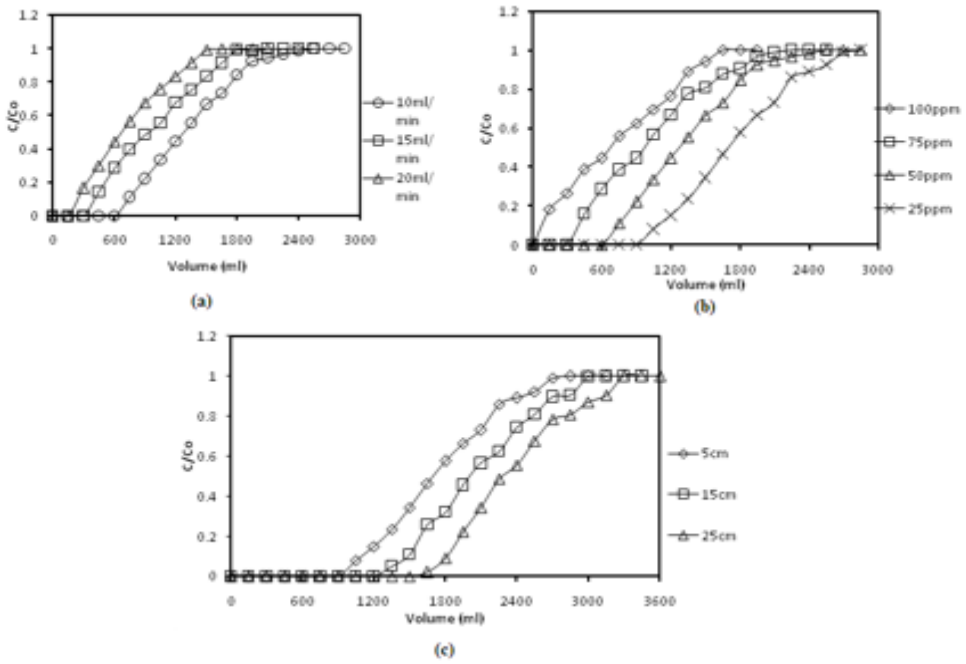


Fig 9: Effect of flow rate, initial metal concentration and bed height for immobilized beads

a=(Initial concentration of lead= 50ppm, bed height = 5cm, Room temperature, pH=5)

b=(Flow rate = 10ml/min, bed height = 5 cm, Room temperature, pH=5)

c=(Flow rate = 10ml/min, Room temperature, pH=5, Initial metal concentration = 25ppm)

3.6.3 Effect of bed height

Figure 9(c) shows the break through curve for effect of bed height on the removal of lead ions. Maximum removal takes place at a bed height of 25cm. The removal percentage increased by increasing the bed height from 5 to 25cm. This may be due to the relatively increasing amount of beads used. The number of viable cells also increases with the increase in immobilized beads, which increases the removal percentage. Thus finally the immobilized beads were able to treat 1500ml of the influent solution and discharge it as metal free solution.

Break through curves for uranium biosorption by Ca-alginate immobilized T. harzianum biomass at different column bed heights were obtained at 5ml/min flow rate and 50mg/L influent uranium concentration. Maximum removal was observed at bed height of 25cm compared to that at 20 and 15cm [18].

4. CONCLUSION

This study showed that the yeast Kluyveromyces marxianus was effective in the removal of lead. Batch experiments provided fundamental information regarding optimum pH, inoculum concentration, temperature and maximum removal of lead ions. To overcome the separation problems of using freely suspended biomass form, as well as, mass loss after regeneration of the biosorbent, the biomass was immobilized in the Ca alginate beads. Column experiments were performed in a packed column, as it makes the best use of the concentration difference known to be a driving force for adsorption. The column studies revealed that bed height, flow rate and initial metal concentration affected removal of lead ions. A successful biosorption process not only depends on uptake performance of the biomass, but also on the constant supply of the biomass for the process. Therefore it is preferable to use biomass, which is either an industrial waste or available plenty in nature. Kluyveromyces marxianus is one of the most abundantly available in nature and also as a waste from many industries. Also the cultivation method of yeast is simple and also incurs low production cost. Thus, Kluyveromyces marxianus possesses all intrinsic characteristics to be employed for the treatment of lead bearing industrial effluents.

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