THE EFFECT OF LEAD (II) IONS ON GROWTH AND BIOACCUMULATION OF YEAST

SAI SUBHASHINI S[1], KALIAPPAN S[2]

[1]Assistant Professor ,SRM Institute for Training and Development,Ekkathuthangal,Chennai,India; [2]Professor, Department of Civil Engineering, Anna University, Chennai, India.
Email: subhasenthil.nh@gmail.com

ABSTRACT

The effect of lead (II) ions on the growth and bioaccumulation properties of yeast *Schizosaccharomyces pombe* and *Kluyveromyces marxianus* was investigated as a function of pH and initial metal concentration. The optimum pH for both organism growths was found to be 5. The minimum inhibitory concentration for *Schizosaccharomyces pombe* was found to be 600 mg/l and for Kluyveromyces marxianus was found to be 700mg/l. Although lead ions concentration caused an inhibition effect on the growth of yeast it was capable of removing lead with a specific growth uptake capacity of 11.23 mg g^{-1}at 100 ppm of initial metal concentration in case of Kluyveromyces marxianus and for *Schizosaccharomyces pombe* showed a 4.01mg $g^{-1}$of specific growth uptake at 100 ppm of intial metal concentration.

Keywords : Bioaccumulation, Lead (II), Schizosaccharomyces pombe, *Kluvernomyces marmaxianus* , Specific growth rate

1 INTRODUCTION

The discharge of heavy metal into aquatic ecosystem has become a matter of concern over the last few decades. The pollutants of serious concern include lead, chromium, mercury, uranium, selenium, zinc, arsenic, cadmium, gold, silver, copper, nickel etc. Most of the heavy metals salts are soluble in water and form aqueous solutions and consequently cannot be separated by ordinary physical means of separation. [1] Release of heavy metals in the environment has received increased attention because of the threat to public health. The most commonly used technologies for heavy metal removal include chemical precipitation, ion exchange, activated carbon adsorption and membrane separation processes. Although these procedures tend to be efficient, they are generally expensive and require frequent service attention. Therefore, the need for economical, effective and safe methods for metal removal has resulted in the search for alternative technologies. The presence of toxic metals such as Pb, As, Hg and Cd in the environment is of great concern due to their health implications. Over the years, anthropogenic activities have greatly increased the level of these toxic metals especially in aqueous systems, hence efficient and cost effective methods are being sought for their removal.

Metal accumulative bioprocesses generally fall into one of two categories, biosorptive uptake by non-living, non-growing biomass and bioaccumulation by living cells. In inactivated biomass (biosorption), the microorganisms usually sequester metal through surface bonding only; with active biomass (bioaccumulation), metals are concentrated through a combination of surface reactions, intra and extracellular precipitation, and intra- and extracellular complexation reactions [2]. The phenomenon of metal accumulation in the microorganism will enhance the metal toxicity and in turn reduces the growth of microorganisms. Using growing cultures in bioremoval could avoid the need for a separate biomass production process such as cultivation, harvesting, drying, etc., but the requirements to maintain cell growth. The effectiveness of these processes is usually dependent on the parameters such as temperature, toxicity, oxygen level and availability of nutrients, etc. If the problem of metal toxicity to the growing cell is overcome by the use of metal-resistant organisms, the continually self-replenishing system can be left to run continuously for extended periods [3] [4].

The objective of the present study was to investigate the effect of lead ions on the growth and bioaccumulation properties of yeast *Schizosaccharomyces pombe* and Kluveronomyces marmaxianus as a function of initial metal ion concentration in batch experiments and to evaluate potential yeast for use in bioremediation of Pb, a heavy metal present in effluents from various industries.

2 MATERIALS AND METHODS

2.1 Miroognanism and growth condition

The *Schizosaccharomyces pombe* (MTCC Code 2665), Kluyveromyces marxianus (MTCC Code 95) were used in this study was obtained from Microbial Type Culture and Gene Bank, Institute of Microbial Technology, Chandigarh, India. The composition of the culture medium used were 1% Dextrose, 0.5% peptone, 0.3% yeast extract and 0.3% malt extract. The cells were grown at 30ºC in the growth medium broth with metal (20 mg/L) and without metal. Inoculum concentration of 1% (v/v) was used for the study. Samples were collected at various time intervals and the optical density was determined at 600 nm, spectrophotometrically.

2.2 Media optimization

The media used were Sabouraud, YD, YP, YPL, Maltose, YM and Mineral [5] [6]. Growth yield was characterized by performing a viable cell count. The optimum concentration of the media components that showed maximum cell growth was studied.

2.3 Metal solution

All the reagents were of Analytical Reagent Grade and were prepared in double distilled water. An aqueous stock solution (1000 mg/l) of Pb (II) ions was prepared using Lead Nitrate salt. The residual Lead concentration in the broth was measured using AAS.

2.4 Biomass study

At the exponential phase of the yeast, 10ml of sample was collected and centrifuged at 6000 rpm for 10 minutes. The sample was then serially diluted and the OD was calculated at 600nm. The samples were centrifuged and the supernatant was discarded. From the pre-weight and the post weight and the dry weight of the biomass was calculated. A calibration graph was plotted. The values of optical density measured were correlated with the concentrations of cells, in terms of dry weight of cells per liter of suspension (g/L). [7].

2.5 Minimum inhibitory concentration

Heavy metal resistant bacteria were determined by plate diffusion method. The metal solution was used in varying concentrations ranging from 100 to 700 mg/l. The lowest concentration of the metal, which inhibits the growth of the microorganism, was considered as the Minimal inhibitory concentration (MIC) of the metal against the yeast [8].

2.6 Batch Study

Batch adsorption experiments were carried out by shaking the flasks at 150 rpm using a rotary shaker at room temperature. The samples were centrifuged and the concentration of metals in the supernatant was measured using AAS. The effect of pH on biosorption was investigated in the pH ranges of 3.0–7.0. The effect of the initial metal ion concentration on the biosorption was studied at optimum conditions as described above except that the concentration of metal ions in the adsorption medium varied between 20-500 mg/L. All the experiments are carried out at 30^{o}C with 1% inoculum concentration which was optimized.

3 RESULTS

3.1 Media optimization

The YPL medium produced a maximum yield of 3×10^{10} CFU/ml and 4×10^{10} CFU/ml for *Schizosaccharomyces pombe* and *Kluvernomyces marxianus* respectively. The optimization of the concentration of Lactose, yeast extract and peptone was carried out. The maximum growth yield is obtained with the composition of 3% lactose, 2% yeast extract and 2.5% peptone. The maximum cell density was found to be 7×10^{10} CFU/ml and 9×10^{10} CFU/ml *Schizosaccharomyces pombe* and *Kluvernomyces marxianus* respectively.

3.2 Growth curve

The growth curve of the yeast in YPL medium with metal concentration of 20 mg/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 18hrs. The exponential phase extended from 18 hours to 24 hours. Stationary phase extended till 70 hours. The yeast grown in medium with metal exhibited a higher growth levels than in medium without metal in case of the *Kluvernomyces marxianus* but in *Schizosaccharomyces* it followed the same pattern.

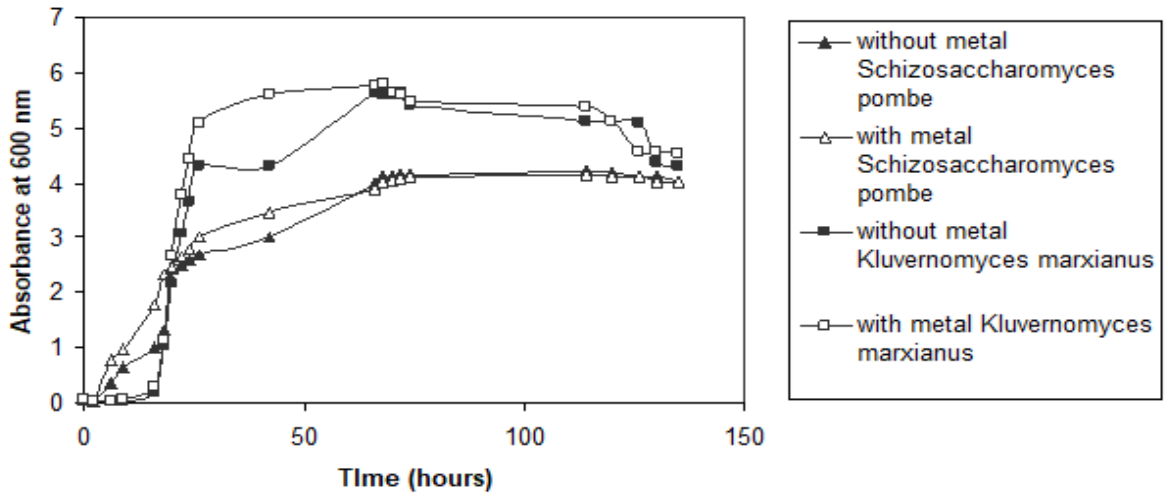

Fig 1. The growth curves of *Schizosaccharomyces pombe* and *Kluyveromyces marxianus*.

3.3 Minimal Inhibitory concentration

The yeast cells were able to grow in growth medium containing the metal concentration of up to 600 ppm for *Schizosaccharomyces pombe* and 700 ppm for *Kluyveromyces marxianus*. Above that no growth was observed.

3.4 Batch study

3.4.1 Effect of pH on the growth and metal ion concentration

pH plays a major role in growth and the lead bioaccumulation properties of yeast . The effect of initial pH on growth and bioremoval is presented in Table 1. The specific growth rate of the microorganism and bioaccumulated quantity of lead (II) increased with increasing pH up to 5.0 and 4.5, respectively. Maximum lead (II) uptake determined as 12.40 mg/ g at this pH values, respectively. No significant activity was observed at the lower and higher pH values. At higher pH values metal ions precipitated because of the high hydroxyl ions concentrations in the medium [9].

TABLE 1 THE EFFECT OF INITIAL PH ON THE MICROORGANISM GROWTH RATE AND MAXIMUM LEAD (II) UPTAKE PER G OF DRIED MICROORGANISM

Organisms	***pH***	***μ (h^{-1})***	***Qm (mg/g)***
Schizosacchaomyces pombe	3	0.0212	5.71
	4	0.0274	9.10
	5	0.0326	11.1
	6	0.0274	7.14
	7	0.0161	7.24
Kluvernomyces marxianus	3	0.011	3.25
	4	0.016	11.35
	5	0.027	12.40
	6	0.01	9.03
	7	0.008	2.28

3.4.2 Effect of initial ion concentration on growth and metal ion bioaccumulation

The initial metal ion concentration in the feed medium was varied in the range 20- 500 mg/l for lead (II) at constant media concentration. *Schizosaccharomyces pombe* showed less resistance to lead (II) and the lowest growth and uptake yields when compared with the *Kluyveronmyces marxianus*. Due to the lead (II) inhibition effect, the specific growth rate and maximum microorganism concentration decreased with increasing lead (II) concentration from 20 to 500 mg/l. The increase of lead (II) concentration also decreased the lead (II) uptake yields. The bioaccumulation capacity of the microorganism increased up to 19.51 mg/g with 20 mg/l of lead (II) concentration in *Kluyveronmyces marxianus.*

TABLE.2 THE COMPARISON OF THE MICROORGANISM GROWTH RATES AND MAXIMUM SPECIFIC LEAD (II) UPTAKES YIELDS OBTAINED AT DIFFERENT INITIAL LEAD (II) CONCENTRATION

Organisms	***Initial metal Concentration (mg/l)***	***μ (h^{-1})***	***Qm (mg/g)***
Schizosacchaomyces pombe	20	0.0163	8.34
	40	0.0108	6.40
	60	0.0089	5.37
	80	0.008	4.89
	100	0.0077	4.01
	200	0.0074	3.62
	300	0.0068	3.11
	400	0.0067	2.10
	500	0.0042	1.13
Kluvernomyces marxianus	20	0.025	19.51
	40	0.025	15.51
	60	0.0225	13.22
	80	0.022	12.89
	100	0.018	11.23
	200	0.014	10.01
	300	0.01	8.89
	400	0.008	6.43
	500	0.006	3.11

3.4.3 Microbial growth and metal ion consumption kinetics

Growth and metal ion accumulation curves of *Schizosaccharomyces pombe* cultivated on 0, 20,40,60,80,100 mg/l of initial lead ion concentration are given in figure 2. A significant decrease in the microorganism concentration was observed for all concentration of lead (II). 8.34mg/g of bioaccumlated lead (II) per g of dried weight at the end of microbial growth was observed in case of *Schizosaccharomyces pombe* and 19.51mg/g of bioaccumlated lead (II) per g of dried weight at the end of microbial growth was observed in case of *Kluvernomyces marmaxianus.*

The figure 2 & 3 illustrates that the growth of the organism decreased with the increase in lead (II) ions concentration. The inhibition effect increased on raising the level of copper (II) in the growth medium and caused a reduction in the final biomass production. K.marxianus growth was sensitive to high concentration of lead (II) with an extension in lag phase duration correlated a decrease in yeast

production.The increase of the lead (II) concentration lead to a drastic decrease of microbial growth and lead (II) uptake for S.pombe.

Metal ion uptake in yeasts is known to involve an initial rapid binding of metal ions to negatively-charged sites on the wall which is a multi-laminate, microfibrillar structure consisting of up to 90% polysaccharide (biosorption) followed by a slower, energy-dependent entry. The outer mannan-protein layers of the yeast cell wall as well as the inner glucan-chitin layer are important heavy metal accumulation. In eukaryotic microbes, a majority of intracellular metals are bound to polyphosphate granules localized in or close to the vacuoles. Intracellular binding and metal detoxification is also mediated by specific low-molecular weight proteins, namely metallothioneins and phytochelatins. Another metal deposition mechanism can be suspected based on the active transport of metal ions [10, 11, and 12].

The studied yeasts are considered to be more effective for concentrating lead (II) ions at different capacities due to lead (II) ion level. Differences between yeast species in the magnitude of change in metal ion uptake capacity may be due to the properties of the yeast (e.g. cellular physiology, structure, surface area, permeability of the cell membrane depending on the yeast division, genera and species). High metal resistance could be attributed to metallothionein and for other metal-induced small protein quantities in *K. marxianus* [12].

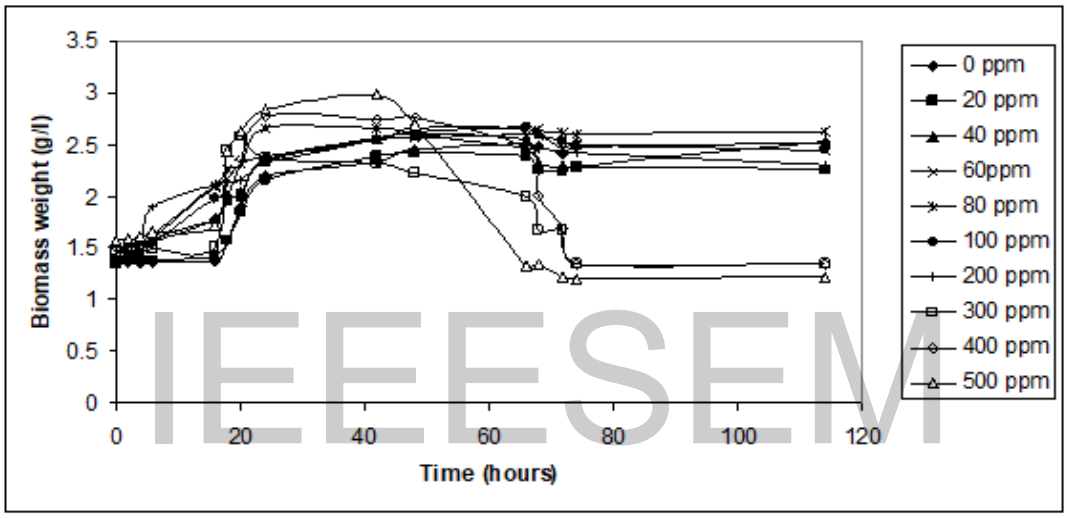

Fig 2 The growth curves of *Schizosaccharomyces pombe* in different metal ion concentration

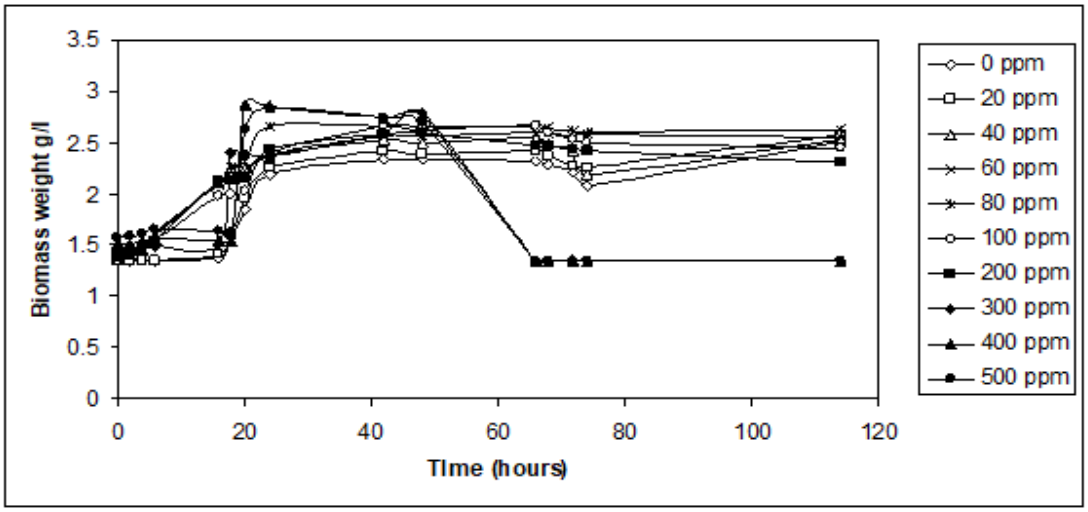

Fig 3.The growth curves of *Kluyvernomyces marmaxianus* in different metal ion concentration

4. Conclusion

The aim of the present work was to examine the bioaccumulation characteristics of *Schizosaccharomyces pombe* and *Kluvernomyces marmaxianus* for the removal of lead (II) ions during growth. The metal biocaccmulation was dependent on the pH and initial metal ion concentration. Although the concentration of the metal causes inhibition in growth of the both organisms, it is highly resistance to lead (II) and could accumulate metal ions at high yields. The present study shows that the *Kluvernomyces marmaxianus* appears to be useful as a living biosorbent for removal of lead ions from wastewater than the *Schizosaccarhomyces pombe*. The result showed that over long period heavy metal adapted cells of yeast are able to bioaccumulate heavy metal cations in high concentration and to remove them from the cultivation suspension.

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