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Lead bioremediation with *Saccharomyces bloulardii*

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Abstract---The main routes of exposure to lead are eating food, adsorbing the skin, drinking water, and breathing the air. This exposure causes many of human health problem. Bioremediation is an ecofriendly, effective and has greater public acceptance. The bioremediation process was carried out using *Saccharomyces bloulardii* yeast and lead at concentration of (0.1,0.2,0.3 mg/ml) under PH=7 condition at 35°C and for a period (24,48,72 hours), and anatomic absorption spectroscopy was used to analyze the concentration of lead after the completion of the treatment process. The results indicate that the highest removal of lead was % at 0.1 mg/ml and lowest removal by 0.2 mg/ml, and the best elimination period was at 72 hours.

Keywords---*Saccharomyces bloulardii*, Lead, Bioremediation, ITS r DNA.

Introduction

The problem of pollution is one of the global environmental problems, the human and rapid request of industrial for economic development and other ways are causes a pressure on natural resources (Megharaj *et al.*,2011) Among the most important pollutant are heavy elements in environment which have caused the accumulation, toxicity and form non-degradable chemical complexes (Paknikar *et al.*,2003).Lead is one of the most toxic elements , Throughout our environment some amount of lead is found as tetra ethyl lead, tetra methyl lead and inorganic lead (EU.Commission,2002). An increased in concentration of lead in the environment comes from human activities including burning fossil fuels, mining, and manufacturing. Due to that heavy elements continue in the environment and are non-degradable, there is a global need to improve sustain the quality of the

environment by friendly option (Sekhar *et al.*,2004). Bioremediation is the process that utilizes living organisms, mainly microorganism, their enzymes, and green plant, to degrade, mineralize, transform, and remove the environmental pollutants and toxic components of the environmental waste. (Azubike *et al.*,2016). Among the various biological treatment options is the use of *Saccharomyces bloulardii* is being used as biomass to adsorption of many heavy metals from aquatic systems, (Podgorskii *et al.*,2004). This research aims to determine the efficiency of *Saccharomyces bloulardii* yeast in bioremediation and reducing lead contamination.

Materials and Methods

Preparaion of yeast isolated

1gm of yeast was weighed and added to 20 ml of Sabourd broth agar media then incubation at 37°C for 24 hours. Where turbidity was observed. based identification ITS r DNA: ITS r DNA was detected through DNA extraction from yeast isolates. Prefixes F (TCCGTAGGTGAACCTGCGG) were used R (TCCTCCGCTTATTGATATGC) manufactured by (Macrogen, South Korea). The (Macrogen) company identified *Saccharomyces bloulardii* isolate. Then the nucleotide sequence was compared with the data available at the NCBI website (<http://blast.ncbi.nlm.nih.gov/> Blast. c gi) Then, a phylogenetic tree was drawn for isolate after matching them with strains in the gene bank and using Mega software. Preation of mineral solutions An aqueous solution of $PbCl_2$ Lead chloride was prepared at concentrations of 100 mg/ml. This solution was used for the number solutions required Etorki *et al.*, (2013). Bioremediation Protocol Flasks were prepared containing 100 ml of Saburaud Dextrose Broth and concentrations were added (0.1,0.2,0.3mg) of lead chloride individually and the PH adjusted to 7, then distributed in tubes of 10ml and adding 0.5ml of yeast growth to each tube so that (it contains 103 cells) and the tubes were incubated at 37°C for a period of (72,48,24 Hour). Hietala and Roane, (2009). After the incubation period was over, they were centrifuged at 6000 rpm for 15minutes, then filtered, and the concentrations measured by atomic absorption spectrophotometer (Philip *et al.*,2000).

Results and Discussion

Diagnosis of *Saccharomyces bloulardii*

Based on Preparation of yeast isolated steps and yeast diagnosis, *Saccharomyces bloulardii* were obtained and according to the apparent diagnosis, colonies of yeast appeared after their growth on Saburaud Dextrose Broth medium, white to creamy in shape with regular edges and smooth, as shown in Fig. (1) (Ellis,1994).



Figure (1): Phenotype of *Saccharomyces boulardii*

Molecular diagnostics

The results of electrophoresis showed on Agarose gel of DNA samples extracted from *Saccharomyces boulardii* isolates, and by using of the primer of ITS r DNA, the DNA bundles were up to 500 base pairs in Figure (2).



Figure 2: Electrophoresis of Agarose gel showing PCR product analysis of ITS r DNA gen in *Saccharomyces boulardii*

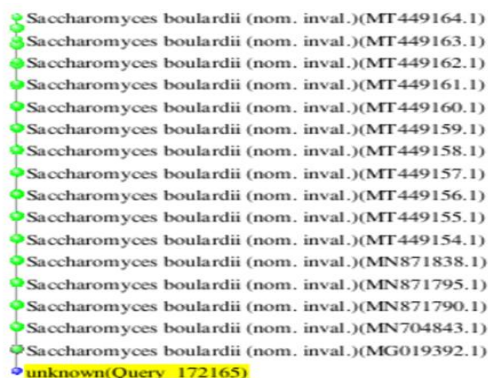


Figure (3) phylogenetic tree analysis based on the ITS r DNA partial sequence of local *Saccharomyces boulardii*. Isolates that used for genetic species identification analysis. The phylogenetic tree was constructed using MEGA 6.

Experiment bioremediation

Table (1,2), the results showed that the yeast have ability to remove lead at a concentration of 0.1 mg/ ml at (0.021) and lowest removal concentration is 0.3 mg/ ml at (0.236). The first stage of metal ion binding in the cells of microorganisms depends on the metabolism of microbes. This stage includes the chemical absorption of ions through the components of the cell wall, and therefore the efficiency of the biological absorption of heavy metals by microbial biomass it is mainly associated with the structure of the cell wall of the microorganism and therefore the process depends on the characteristics of the cell surface in which it determines the normal structure of the interaction between the microorganism and the heavy metal (Fraile P, *et al.*, 2000), (Hernandez, P, *et al.*, 2006), the negatively charged yeast cell wall and the ability of the yeast cell wall to bind to heavy metal ketones is likely due to electrostatic interactions (Tominaga T, *et al.*, 1996), where microbes can absorb heavy metals through the binding sites in the cellular structure without exchanging energy, and among the reactive compounds attached to the cell wall are extracellular polymeric materials such as exopolysaccharides or Exopolysaccharide (EPS), which have a significant effect on acidity. And the basicity of the middle as well as the great ability to complex heavy metals (Tominaga T, *et al.*, 1998), while monosaccharides and acidic components are good binding agents as well as confer high capacity for cell surface biosorption. Biosorption depends on many factors, the most important of which are cell wall formation, biomass growth, nutrition, age and product composition. Cellular exterior (Luksiene Z, *et al.*, 2013), where it was found that cell wall composition and polysaccharide content can vary by more than 50% depending on the nature of the carbon source and nitrogen limitation as well as temperature and ventilation and thus affects the transport of minerals.

Table (1) results of yeast activity. *S. boulardii* was used to remove Lead in concentrations (0.1, 0.2, 0.3mg/ml) within (24, 48, 72) hours

	Time 24	Time 48	Time 72	P-value	LSD
Control	0.217	0.217	0.217	0.547	0.040
0.1	0.073	0.061	0.021	0.547	0.040
0.2	0.178	0.132	0.095	0.547	0.040
0.3	0.236	0.200	0.137	0.547	0.040

Conclusion

We concluded from this study that the results of our experiment indicate that the highest removal of lead was % at 0.1 mg/ml and lowest removal by 0.2 mg/ml, and the best elimination period was at 72 hours.

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