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Cadmium biological treatment with *Saccharomyces bloulardii*

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Abstract---The bio removal process was carried out using *Saccharomyces bloulardii* and cadmium at concentrations (0.1, 0.2, 0.3 mg/ml) at temperature of 37°C and PH7 for a period (24, 48, 72 hours). The results showed that the highest of cadmium removal at concentration of 0.1 mg/ml. Also, it has been study of effect of incubation period on removing heavy element ions and it was found that the best period was 72 hours compared to 48 and 24 hours.

Keywords---cadmium biological treatment, *Saccharomyces bloulardii*, removal process.

Introduction

The heavy metals have an atomic number greater than 20 and atomic density above 5g/cm³, (Forester and Wase, 1997). The toxic elements are naturally occurring elements that comprise essential (Fe, Cu, Ni, Zn, Mg) and nonessential (Cd, Hg, Pb, As) (Al Hejuje, 2014). The heavy metals are present at basic levels of natural origin from the weathering process of the rocks (Facchine II *et al.*, 2001) and they are present by human activities such as industrial, agricultural, and mining processes that led to a widespread of heavy metals in the ecosystems. (Al. Shammari, 2013). These elements are danger on human health and all living organisms (Kim *et al.*, 2007). The risks of heavy metals on health depending on the level and duration of exposure: Acute exposure: Exposure to large amounts of heavy metals in a short period of time. Chronic exposure: Exposure to small amounts of heavy metals over a long period of time (Young, 2000). Cadmium is a heavy and highly toxic element. It is the element found in nature in the form of oxides, sulfates, or chlorides. It is considered one of the constituent elements of the earth's crust. The atomic number of cadmium is 48 (Atsdr, 1999). When humans or animals are exposed to this metal, it accumulates in their bodies for

life Bernard,2008). Cadmium is used in many industries such as phosphate fertilizers, refining processes and nickel-cadmium rechargeable batteries (Gil *et al*, 2011), Cadmium is one of the most toxic heavy metals, as its danger increases after its accumulation in the body to cause many health problems such as osteoporosis, heart enlargement, high blood pressure and lung cancer (Toman *et al*, 2005). Cadmium has natural and other human sources. The natural ones include volcanic activity, forest fires, and the transfer of soil particles into the air (Patra *et al*, 2001). As for humankind, it is produced by various industries such as mining, smelting, tobacco smoking, waste burning, and others Cadmium is classified according to the Environmental Protection Agency (EPA) as one of 129 pollutants and is one of the 25 dangerous substances that threaten life (WHO, 1996). With regard to its effect on plants, it causes problems in physiological and biochemical processes in plant tissues, such as nitrogen fixation and transport (Martin & Griswold, 2009).

Materials and Methods

Preparation of yeast isolated of yeast was weighed and added to 20 ml of Sabouraud 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.cgi>) 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 $CdCl_2$ cadmium 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 cadmium 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).

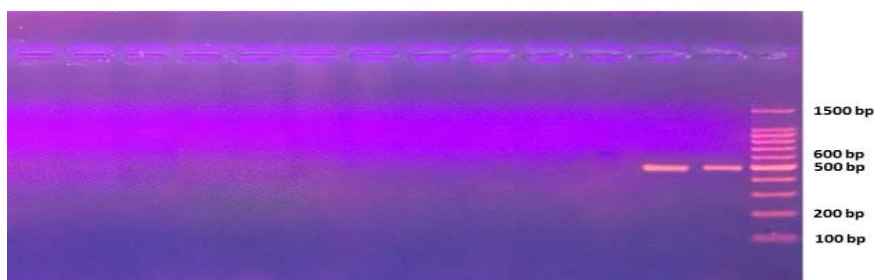


Figure 2: Electrophoresis of Agarose gel showing PCR product analysis of ITS r DNA gen in *Saccharomyces boulardii*

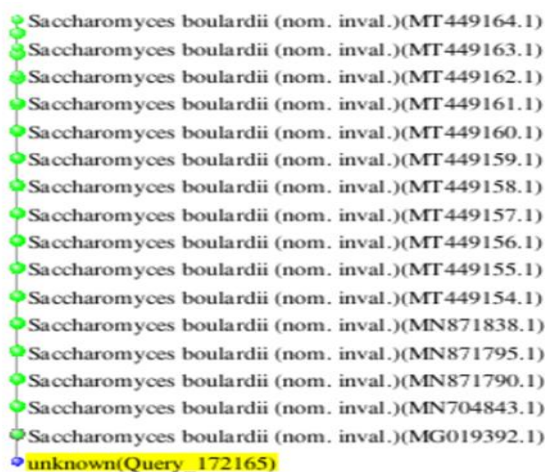


Figure (3) phylogenetic tree analysis based on the ITS r DNA partial sequence of local *Saccharomyces boulardii*. Isolates that used for genetic species identification analysis. The phylogenetic tree was constructed using MEGA 6

Experiment bioremediation

The table (1) the results showed that the yeast has ability to remove Cadmium at a concentration of 0.1 mg/ ml at (0.063) and lowest removal concentration is 0.3 mg/ ml at (0.218) 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 cadmium in concentrations (0.1, 0.2, 0.3mg/ml) within (24, 48, 72) hours

	Time 24	Time 48	Time 72	P-value	LSD
Control	0.222	0.222	0.222	0.681	0.030
0.1	0.069	0.066	0.063	0.681	0.030
0.2	0.182	0.149	0.138	0.681	0.030
0.3	0.212	0.218	0.213	0.681	0.030

Conclusion

We concluded from this study that the results showed that the highest of cadmium removal at concentration of 0.1 mg/ml. Also, it has been study of effect of incubation period on removing heavy element ions and it was found that the best period was72 hours compared to48 and 24 hours.

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