Bioremediation of some types of heavy metals by *Candida* spp.

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***Abstract*— It was isolated and diagnosis of 109 isolates (*C. tropicalis*) and 85 isolates (*C. glabrata*) , isolated from water and soil Basra/ Iraq . These isolates have shown its ability to get rid of the bioremediation of environmental pollutants such as heavy metals (lead , cobalt, and cadmium) , as they have the ability to removal or bio – accumulation rates different from those elements. And during the current study proved that the time has an important role increasing the bioremediation any rate the greater the bosom of microbiology period including yeasts in rural contaminated increased disposal of environmental pollutants and vice versa ratio.**

Index Terms—**Bioremediation , yeasts, heavy metals.**

I. INTRODUCTION

Bioremediation is an integrated management of polluted ecosystem where different microorganisms are employed which catalyze the natural processes in the polluted or in the contaminated aquatic or terrestrial ecosystem. Suitable, but high cost technologies have been identified for cleanup of heavy metal polluted soils (Iskandar and Adriano, 1997). Bioremediation generally utilizes microbes (bacteria, fungi, yeast ,and algae), but higher plants are used in some applications. Although the bindings of metals to microorganisms have been described for many years, the commercial use of this procedure is slow. Microorganisms (bacteria, yeast and protozoa) showed remarkable ability to pick up heavy metals from the culture medium when they were used individually. Heavy metals in the environment have been prioritized as major inorganic contaminants due to their recalcitrance and consequent persistence (Atkinson *et al.*, 1998). The main sources for heavy metal contamination are mining activities and industrial wastewaters, discharging a variety of toxic metals such as Cd, Cu, Ni, Cr, Hg, Zn and Pb into the environment (Soares*et al.*, 2003; Malik, 2004).

Traditional technologies for heavy metals removal such as chemical precipitation, ion exchange, or reverse osmosis processes are very expensive and have several disadvantages, such as unpredictable metal ion removal , high reagent requirements and generation of toxic sludge, which are often difficult to dewater and require extreme caution when disposing of them (Siloniz*et al.*,2002a). New technologies, like biosorption, are required to reduce heavy metal concentrations to acceptable environmental levels at low costs. Microorganisms may be used to remediate wastewaters or soils contaminated with heavy metals. The metal processing capacity of microorganisms can be used to concentrate, remove and recover metals from aqueous streams and enhance the efficiency of wastewater treatment processes (Amoroso *et al*., 1998). They have proven capability to take up heavy metals from aqueous solutions, especially when the metal concentrations in the effluent range from less than 1 to about 20 mg/L (Brierley, 1990).
In a biotechnological context, yeasts may be useful in the metal-containing effluents treatment(Blackwell *et al*., 1995). Metal accumulation bioprocesses generally fall into one of the two categories, biosorptive uptake by non-living or nongrowing biomass and bioaccumulation by living cells (Akzu and Donmez, 2001). Active uptake systems can take up both essential and non-essential metal ions and thus are of interest in bioremoval. The essential characteristics of a living biomass used in a metal ion removal process are tolerance and uptake capacities (Macaskie and Dean, 1989; Aksu, 1998; Suh, 1998). One of the most ubiquitous biomass types available for bioremediation of heavy metals at low pH is yeast. Yeast biomass is an inexpensive ,readily available source of biomass. Furthermore, yeast cells retain their ability to accumulate a broad range of heavy metals to varying degrees under a wide range of external conditions.

In this study different combinations of microorganisms were used to evaluate the best combination for efficient removal of heavy metals.

1- Species of isolates :
   109 isolate to the yeast *Candida tropicalis* and 85 isolate to yeast *C. glabrata* . from water and soil of environmental of Basrah / Iraq.

II. Materials and Methods

Yeast Isolates: Two species of *Candida* viz *C. tropicalis* and *C. glabrata* previously isolated from water and sediments of Basrah water bodies (unpublished data) were used in this study. Yeast grown on PDA medium and store in refrigerator until used.

Preparation of heavy metals concentration:
A standard solution of the three elements (Pb, Co, Cd) was prepared in order to dissolving the weight of 250 mg for each element in 250 ml sterile ion free D.W. Three concentrations of each element were prepared where the concentrations of element lead and cobalt is (1,5,10) mg/l , while cadmium is (1,1.5,2) mg/l.

Biosorption test studies: The batch biosorption experimental method was used to determine the sorption of the each heavy metal by the various isolates obtained . specific weights 0.5g of the respective biomass were introduced into 10 ml of the each heavy metal concentration

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contained in a 100ml Erlenmeyer flask for 24h at room temperature on a rotary shaker at 120 rpm . At the end of incubation duration , the biomass was separated by centrifugation at 4000 rpm for 30 minutes and supernatants were analyzed for residual metal concentration using an atomic absorption spectrophotometer . the sorbet or heavy metal uptake [q (mg metal / g dry cells)] was calculated . (Volesky,1995). As:

$$Q = \frac{V(L) * (Ci - Cf)(mg / l)}{}$$

Where ,
Q= heavy metal uptake
V= volume of metal solution
C_i= initial concentration of metal in solution
C_f= final metal concentration in solution
S= mass of dried cells

III. Results

The results showed the ability of some species of yeasts isolated (*C. tropicalis* & *C. glabrata*) on biological treatment to remove environmental pollutants or heavy metals (Pb , Co, Cd) that present in water and sediments. These species were involved in the process of bio – accumulation of these elements , and it was found through statistical analysis at the level of probability 0.05.

Removal of lead (Pb)

As shown in figure (1) the effect of time on the proportion of removal of various concentrations of the heavy element lead by *C. tropicalis* and the highest percentage (2%) of removal were observed at the time of 48 hrs to a concentration 10mg/l. Lowest rate (0.6%.) for the removal of concentration is at concentration 1mg/l at the time of 24 hrs.
As shown in figure (2) the effect of time on the proportion of removal of various concentrations of the heavy element lead by *C. glabrata* and the highest percentage (1.9%) of removal were observed at the time of 48 hrs to a concentration 10mg/l. Lowest rate (0.6%.) for the removal of concentration is at concentration 1mg/l at the time 24 hrs.
As shown in figure (2) the effect of time on the proportion of removal of various concentrations of the heavy element lead by *C. glabrata* and the highest percentage (1.9%) of removal were observed at the time of 48 hrs to a concentration 10mg/l. Lowest rate (0.6%.) for the removal of concentration is at concentration 1mg/l at the time 24 hrs.

Biosorption test studies: The results showed the percentage of heavy metal uptake inside the yeast cells.
The highest concentration of absorbance of lead at 10mg/l was 1.1% when yeast isolates were mixed. The case of less absorbance of lead 0.012%. was observed by *C. glabrata* at 1mg/l. Figure (4).
Regarding the cobalt element as shown in figure (5) the effect of time on the proportion of removal of various concentrations of the heavy element cobalt by *C. tropicalis* and the highest percentage (6.5%) of removal were observed at the time of 48 hrs to a concentration 10mg/l. Lowest rate (0.2%.) for the removal of concentration is at concentration 1mg/l at the time 24 hrs.

As shown in figure (6) the effect of time on the proportion of removal of various concentrations of the heavy element cobalt by *C. glabrata* and the highest percentage (6.3%) of removal were observed at the time of 48 hrs to a concentration 10mg/l. Lowest rate (0.2%.) for the removal of concentration is at concentration 1mg/l at the time 24 hrs.
As figure (7) has the effect of time on the proportion of said removal of various concentrations of heavy element cobalt by mixed isolates of *C. tropicalis* & *C. glabrata* showed highest percentage of removal at the time of 48 hrs. to a concentration of 10mg/ml the 6.6% , lowest rate for the removal of concentration is 1mg/l the 0.5% at the time 24 hrs.

Biosorption test studies: The results showed the percentage of heavy metal uptake inside the yeast cells.
The highest concentration of absorbance of cobalt at 10mg/l was 0.2% when yeast isolates were mixed. The case of less absorbance of lead 0.02%. was observed by *C. glabrata* at 1mg/l. Figure (8).
Regarding the cadmium element as shown in figure (9) the effect of time on the proportion of removal of various concentrations of the heavy element cadmium by *C. tropicalis* and the highest percentage (2.8%) of removal were observed at the time of 48 hrs to a concentration 2mg/l. Lowest rate (1.2%.) for the removal of concentration is at concentration 1mg/l at the time 24 hrs.
As shown in figure (10) the effect of time on the proportion of removal of various concentrations of the heavy element cadmium by *C. glabrata* and the highest percentage (2.8%) of removal were observed at the time of 48 hrs to a concentration 2mg/l. Lowest rate (1.3%.) for the removal of concentration is at concentration 1mg/l at the time 24 hrs.
As figure (11) has the effect of time on the proportion of said removal of various concentrations of heavy element cadmium by mixed isolates of *C. tropicalis* & *C. glabrata* showed highest percentage of removal at the time of 48 hrs. to a concentration of 2mg/ml the 4.1% , lowest rate for the removal of concentration is 1mg/l the 1.4% at the time 24 hrs.

Biosorption test studies: The results showed the percentage of heavy metal uptake inside the yeast cells.
The highest concentration of absorbance of cadmium at 2mg/l was 0.3% when yeast isolates were mixed. The case of less absorbance of lead 0.1%. was observed by *C. glabrata* at 1mg/l. Figure (12).

IV. Helpful Hints

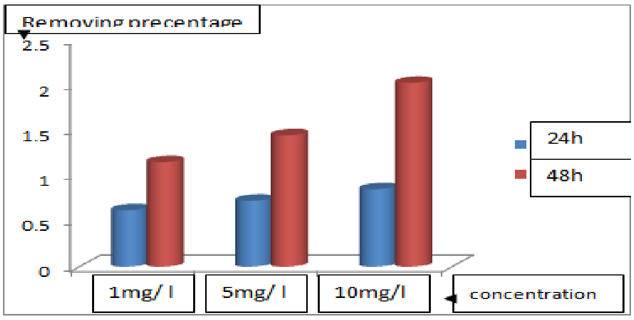

Figure (1) The effect of time on the effectiveness of the removal of lead element by *C. tropicalis*

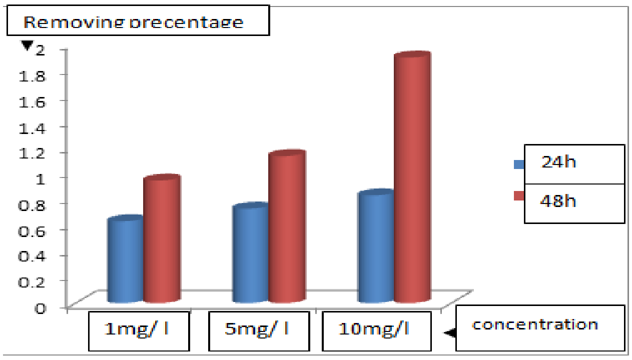

Figure (2) The effect of time on the effectiveness of the removal of lead element by *C. glabrata*

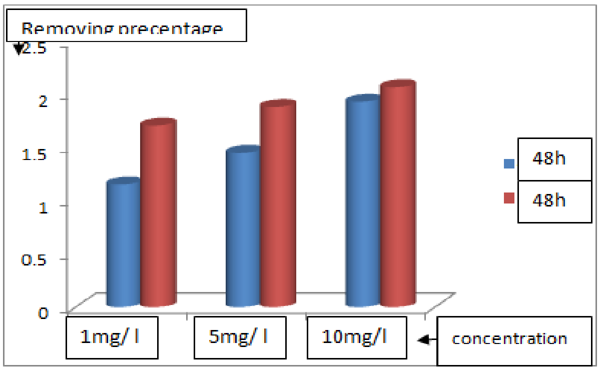

Figure (3) the effect of time on the effectiveness of the removal of mixed isolates of *C. tropicalis* & *C. glabrata* in different ratios of different concentration of the element lead.

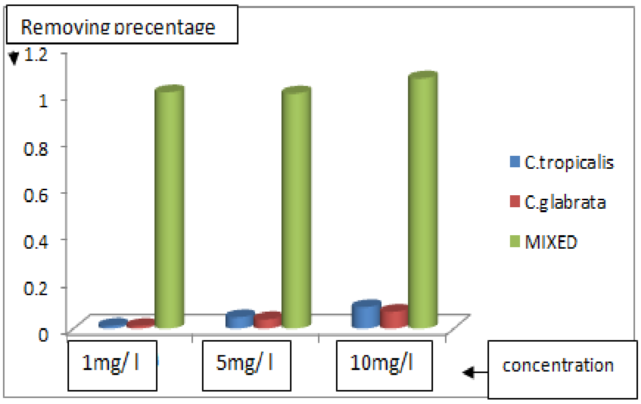

Figure (4) Uptake of heavy elements (Pb) by yeast cells at different concentrations.

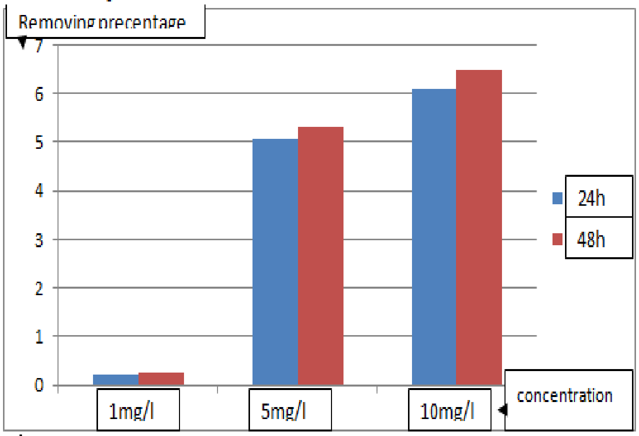

Figure (5) The effect of time on the effectiveness of the removal of cobalt element by *C. tropicalis*

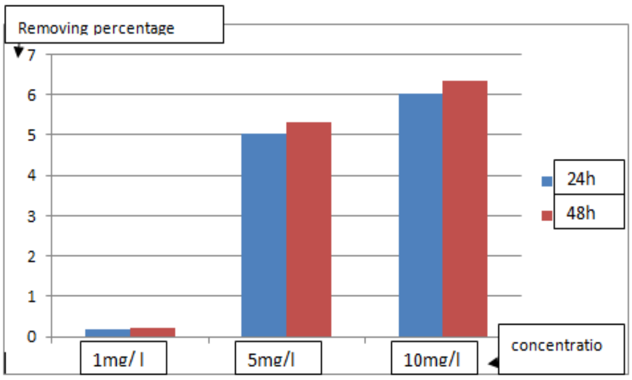

Figure (6) The effect of time on the effectiveness of the removal of cobalt element by *C. glabrata* .

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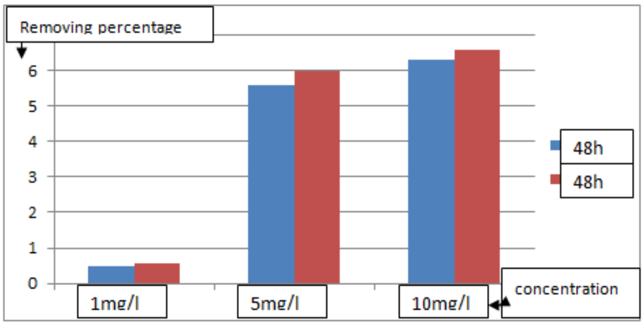

Figure (7) the effect of time on the effectiveness of the removal of mixed isolates of *C. tropicalis* & *C. glabrata* in different ratios of different concentration of the element cobalt.

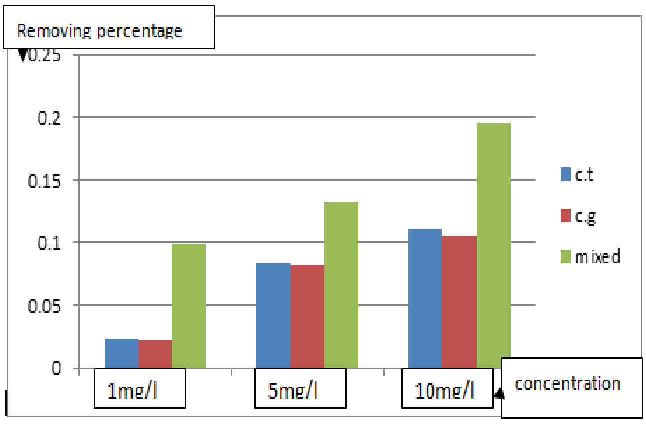

Figure (8) Uptake of heavy elements (Co) by yeast cells at different concentrations.

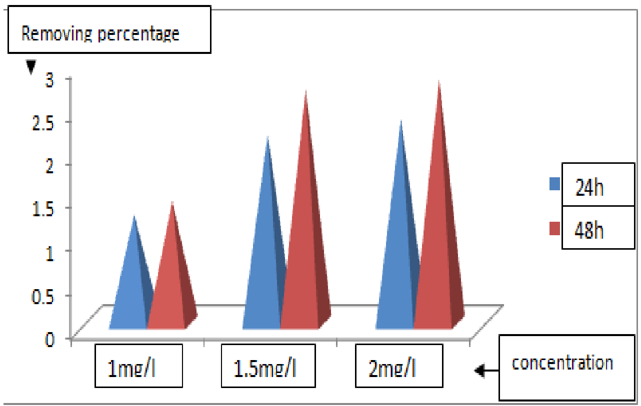

Figure (9) The effect of time on the effectiveness of the removal of cadmium element by *C. tropicalis* .

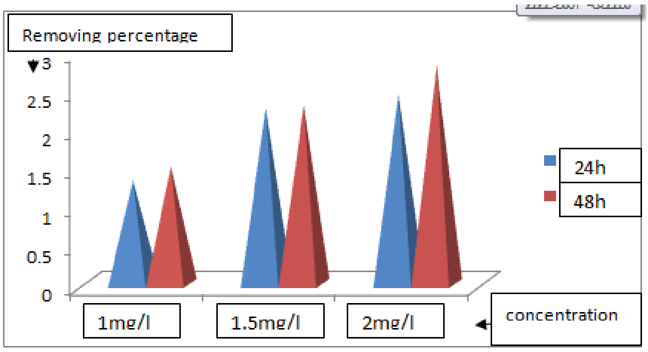

Figure (10) The effect of time on the effectiveness of the removal of cadmium element by *C. glabrata* .

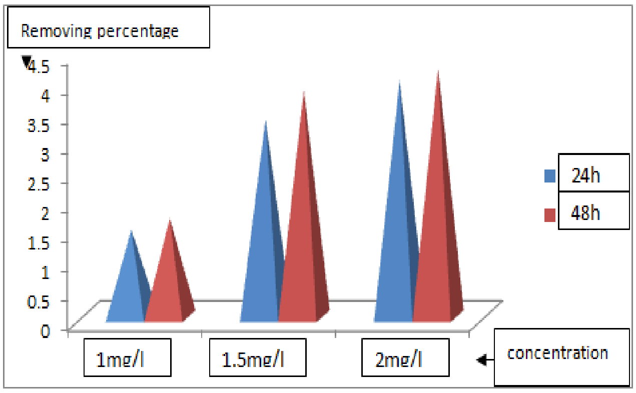

Figure (11) the effect of time on the effectiveness of the removal of mixed isolates of *C. tropicalis* & *C. glabrata* in different ratios of different concentration of the element cadmium.

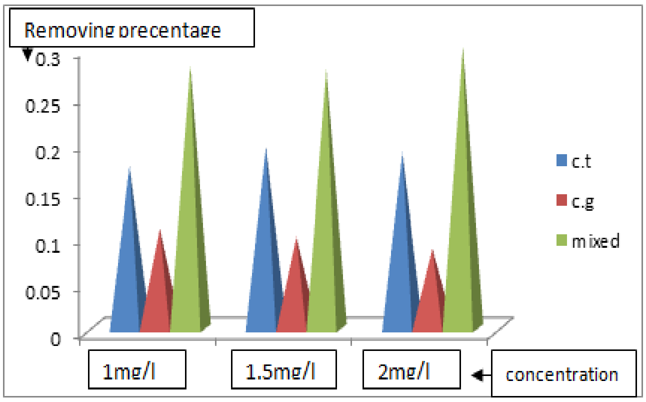

Figure (12) Uptake of heavy elements (Cd) by yeast cells at different concentrations.

V. Discussion

A large variety of microorganisms including bacteria, yeasts and protozoa are found in industrial wastewater (Haq and Shakoori, 2000; Rehman and Shakoori, 2001,2003).
Several studies have reported improvements in metal removal by immobilization of protozoa, yeast or bacterial cells (Zeroual *et al.*, 2001).Bacteria and yeast communities are central to the functioning of terrestrial ecosystem and consist of a large number of different bacterial and yeast type (O-Muter *et al.*, 2002).
The principal goal of bioremediation is to enhance the natural biological-chemical transformations that render pollutants harmless as minerals and thus to provide a relief and, if feasible, a permanent solution to the problem of contaminated environments. Remediation of sites contaminated with heavy metals is a complex problem (Sandrin *et al.*, 2000; De *et al.*, 2006). Bioremediation can be effective where environmental conditions permit microbial growth and activity (Vidali, 2001).

It was observed that protozoa may not beimportant in large scale processing of wastes containing heavy metals, but they share the capability of resisting this toxic metal ion with othermicroorganisms like bacteria and yeast. Mixedculture is considered to be important in anecosystem due to cooperative actions. It would notbe advisable to use a pure culture of amicroorganism due to disturbances in populationstructures in an ecosystem.
Removal of heavy metals from wastewater is normally achieved by advance technologies such as ion exchange, chemical precipitation, ultra filtration, or electrochemical deposition do not seem to be economically feasible for such industries because of their relatively high costs. Therefore, there is a need
to look into alternatives to investigate a low-cost method, which is effective and economic, and can be used by such industries. More practical methodsare being explored. One of these methods is toisolate heavy metal resistant microorganisms asthese have evolved strategies to cope up with stressed conditions (Stadler *et al.*, 2004).Bioremediation of heavy metals usingmicroorganisms has received a great attention inrecent years for its potential application in industry ,as it is nondestructive, cheap and economical (Rise- Roberts, 1998; Rehman *et al.*, 2007).

In order to assess the ability of yeast isolates to decrease Cu2+ in contaminated industrial effluentsa mini large-scale experiment was done. Industrial wastewaters harbor a variety of microorganisms including bacteria, fungi, algae and ciliates. *C. tropicalis* was able to remove 64% copper from the wastewater after 4 days and was also capable to remove 74% from the wastewater after 8 days ofincubation at room temperature. Siloniz *et al.* (2002b) described the ability of yeast, isolated from sewage sludge, to take up copper in response to increasing concentrations of this metal in the culturemedium. Moreover Balsalobre *et al.* (2003) indicated that both the tolerance to metals and thecapacity of the uptake are dependent on ionic metal and yeast species.
This is what agreed with the current terms of our study proved susceptibility of yeasts to absorption or bio – accumulation of heavy metals.

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