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Study on Removal of Hexavalent and Trivalent Chromium Ions by Microbial cells

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Abstract: The presence of inorganic pollutants such as metal ions (Cr+6 and Cr+3) could destroy our environment and ecosystem. To overcome this problem, much attention was directed to the microbial technology. Microbial cells could be tolerated to the toxic effects and decreased their concentration with keeping their viability. Therefore, building up a complementary strategy to study the cell population under the stress of heavy metals. As target resistive organisms, Rhizobium-MAP7-MG214656 and Rhodotorula ALT72 were used .

The tested Rhizobium strain showed higher ability of removal heavy metals and more resistive to metals ions. So using the two strains especially Rhizobium-MAP7-MG214656 support the removal of heavy metals (Cr+6 and Cr+3).

keywords: Heavy Metal Contaminants, Heavy Metals analysis, bioremediation

1.Introduction

There are number of pollutants like fertilizers, pesticides, heavy metals which seriously affect the living and non-living materials. Heavy metals such as Fe, Zn, Co, Ni, Cr and Cd are causing specific toxicity symptoms even in low concentrations of about 1.0-10 mg/l because they accumulate in the soft tissues [1-3].

The toxicity of heavy metal may result from alterations of numerous physiological processes caused at cellular/molecular level by inactivating enzymes and blocking functional groups of metabolically important molecules [4]. On the other hand, some heavy metals like Fe, Cu, Zn, Ni and other trace elements are important for biological systems and their deficiency or excess could lead to a number of disorders [5].

Chromium (III) and chromium (VI) have different toxicity characteristics. Cr^{+3} is essential for human nutrition and considered as non-toxic. The Cr (VI) is carcinogenic and toxic even in small amounts which diffuses through the epidermis and reduced to Cr (III) which interacts with nuclear enzymes, proteins nucleotide and DNA [6]. Ingestion of Cr ions in large amounts can cause stomach upsets and ulcers, convulsion, kidney and liver damage and even death [7]. The level of chromium must not be higher than the permissible limit 0.05 mg l^{-1}(ppm).

Bio-monitoring assays detecting the toxicity of heavy metals, pesticides and organic solvents applied for microorganisms [8], bacteria [9]. Microorganisms have biosorption capabilities as well as they are easy to culture in short generation time so their response to toxic substances are quite rapid [10].

Several bacteria are being researched for their ability to absorb heavy metals [11] especially for Cr removal [12] due to their small size, ability to grow under organized conditions and the presence of different functional groups on their cell wall that act as binding sites for the contaminant Cr ions[13].

Soil bacteria *Rhizobium sp*. is one of the major element for the maintenance of soil fertility where it has the ability to fix nitrogen in leguminous plants [14, 15] . *Rhizobium* can be used as an indicator organism to several

toxic chemicals, including heavy metals[16] and used in effective, economical and eco-friendly metal bioremediation technologies [17].

Cell wall components of pink *Rhodotorula rubra* and *Rhodotorula* Y11 is containing chitin, carotenoid pigments, could have active metal sorption sites that able to accumulate cadmium and lead[18-21].

Microbial-based technology for the detoxification of heavy metal in polluted systems an economical and more environmentally friendly remediation option [22]. Bacteria and fungi can develop modified metabolism to deal with environmental contaminants and then be used in bioremediation [23].

Thus, the main concern of this work is to develop a system that support the removal of heavy metals (Cr^{+6} and Cr^{+3}) using a selection of microbial cells

2.M aterials and methods

Microorganisms and growth conditions

Gram-negative, nitrogen fixer soil bacterium *Rhizobium* and unicellular pigmented yeasts *Rhodotorula ALT72* donated from Plant Physiology and Genetics Labs at the Faculty of Science, Mansoura University. Strains were cultured on L.B medium at 28±1^{o}C and 150 rpm. The microbial growth estimated by measuring the optical density at 600 nm wavelength after 48 hours for *Rhizobium*-MAP7-MG214656 after 60-72 hours for *Rhodotorula* ALT72.

Determination of Minimum Inhibitory Concentration (MIC)

Sterilized 25ml LB media amended different concentrations 1, 10, 20, 30, 40 and 50 ppm of Cr^{+6} and Cr^{+3} metal ions inoculated by 100 µl of *Rhizobium*-MAP7-MG214656 and *Rhodotorula* ALT72 and incubated at 28±1^{o}C, 150 rpm. To obtain growth rate, the optical density of each flask was measured after 48 hours for *Rhizobium*-MAP7-MG214656 and 60-72hr *Rhodotorula* ALT72 at 600 nm wavelength. The minimal inhibitory concentration (MIC) is defined as the lowest concentration that causes no visible growth [24].

Turbidimetric determination of spectrophotometer

Cells of *Rhizobium*-MAP7-MG214656 and *Rhodotorula* ALT72 were used for the biosorption of Cr^{+6} and Cr^{+3} ions. A loopful of log phase culture of each strain was inoculated separately in flasks containing LB broth medium amended with different concentrations 0.01, 0.1, 1, 10 and 50 ppm of Cr^{+6} and Cr^{+3} metals. The inoculated flasks were then incubated at 28±1^{o}C and 150 rpm. To obtain growth rate, the optical density of each flask was measured 72 and after 48 hours for *Rhodotorula* ALT72 and *Rhizobium* MAP7-MG214656 respectively at 600 nm [25].

Heavy Metals Analysis

Cr^{+6} and Cr^{+3} heavy metals determined by atomic absorption spectroscopy [26]. Sterilized LB media amended different concentrations 0.01, 0.1, 1, 10 and 50 ppm of Cr^{+6} and Cr^{+3} metals inoculated by 100µl of *Rhizobium*-MAP7-MG214656 and *Rhodotorula* ALT72 and incubated 48hr$_s$ for *Rhizobium*-MAP7-MG214656 and 72 h for *Rhodotorula* ALT72 at 28±1^{o}C, 150 rpm. After incubation periods, cultures centrifuged at 6000 rpm for 6 min. The remaining concentrations of heavy metals in supernatant compared with the basic concentrations of heavy metals before inoculation. The optical density of each flask also measured at 600 nm wavelength.

The results were expressed, as percentage of metal removal by using the following equation:

$$\text{Metal removal (\%)} = (c_i - c_f) / c_i \times 100$$

Where, C_i and C_f stand for the initial and final metal concentrations respectively

3. Results and Discussion

Spectrophotometric Determination of Minimum Inhibitory Concentration (MIC)

The growth rate of the treated microbial cells with different concentrations of heavy metals was monitored using the classical spectrophotometric method. The results showed that the treated *Rhodotorula* ALT72 gives higher growth (i.e. less sensitive to get inhibited by the heavy metals) when the culture was contaminated with Cr^{+6} (1 ppm) and for Cr^{+3} (1 and 10ppm) and moderate growth in concentration (20 ppm) while *Rhizobium-MAP7-MG214656* gives a high growth in (1

ppm) and moderate growth in concentration (10 ppm) of Cr^{+6} and gives a high growth (1 ppm) of Cr^{+3} (Fig. 1)

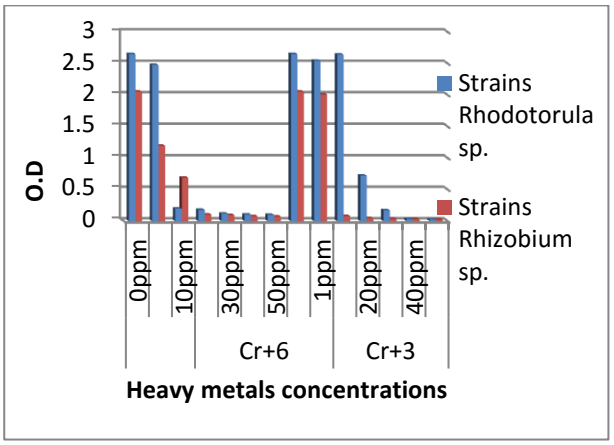

Fig. (1): Spectrophotometric monitoring of the effects of different heavy metal concentrations on the growth rate of microbial cells (*Rhodotorula* ALT72 and *Rhizobium*-MAP7-MG214656). Different concentrations of the metal ions Cr^{+6} and Cr^{+3} (1, 10, 20, 30, 40 and 50 ppm) were utilized.

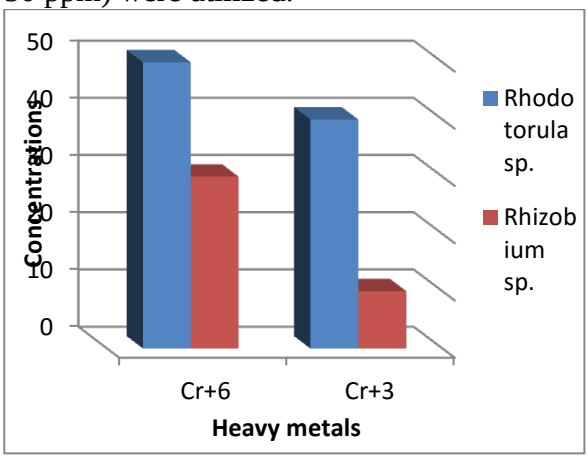

Fig. (2): Sensitivity of *Rhodotorula* ALT72 and *Rhizobium*-MAP7-MG214656 to the determined minimal inhibitory concentrations (MICs) of Cr^{+6}, Cr^{+3}.

Turbidimetric determination of spectrophotometer

Spectrophotometric method was used to detect the growth rate of the treated microbial cells with different concentrations of heavy metals. The results showed that the treated *Rhodotorula* ALT72 gives higher growth (i.e. less sensitive to get inhibited by the heavy metals) when the culture was provided with Cr^{+6} and Cr^{+3} (10 ppm) while *Rhizobium*-MAP7-MG214656 gives a high growth in (10 ppm) and moderate growth in concentration (10 ppm) of Cr^{+6} and gives a high growth (10 ppm) and (1 ppm) of Cr^{+3} (Fig.3)

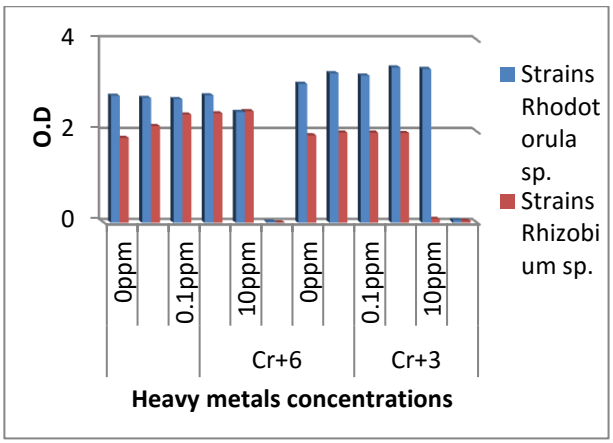

Fig. (3): Spectrophotometric monitoring of the effects of different heavy metal concentrations on the growth rate of microbial cells (*Rhodotorula* ALT72 and *Rhizobium*-MAP7-MG214656). Different concentrations of the metal ions Cr^{+6} and Cr^{+3} (0.01, 0.1, 1and 50 ppm) were utilized.

Consumption of heavy metals by microorganisms

As shown in Table (1), both of *Rhodotorula*ALT72 *and Rhizobium* MAP7-MG214656 were exposed to several concentrations of Cr^{3+} and the uptake rate of heavy metal was analyzed by considering the remaining concentration of the heavy metals in the microbial culture. At the moderate dose 10 ppm, *Rhodotorula*ALT72 was still survived and showed an uptake of Cr ions with 6.47%. However, the *Rhizobium* MAP7-MG214656 uptake with pecentage 2.7% while two strains at lethal dose which is 50 ppm did not show up any capacitance of removal. Regardless the lethal concentration, *Rhizobium*MAP7-MG214656 has higher removal efficiency than the *Rhodotorula* ALT72, whereas the highest removal efficiency was reached 76 % by the *Rhizobium* MAP7-MG214656 compare to 66% by the *Rhodotorula* ALT72 at 0.1ppm.

On the other hand, the microbial removal of Cr^{+6} ions by the *Rhodotorula*-ALT72 and *Rhizobium* MAP7-MG214656 was analyzed over different concentrations. The result showed in Table (1) exhibited the higher efficiency of the Cr^{+6} by the *Rhizobium*MAP7-MG214656 whereas the maxim capacity of removal was about 45% at the concentration of 0.1 ppm. Nevertheless, both strains could not survive at the lethal dose; therefore, zero% of removal was attained using both of them.

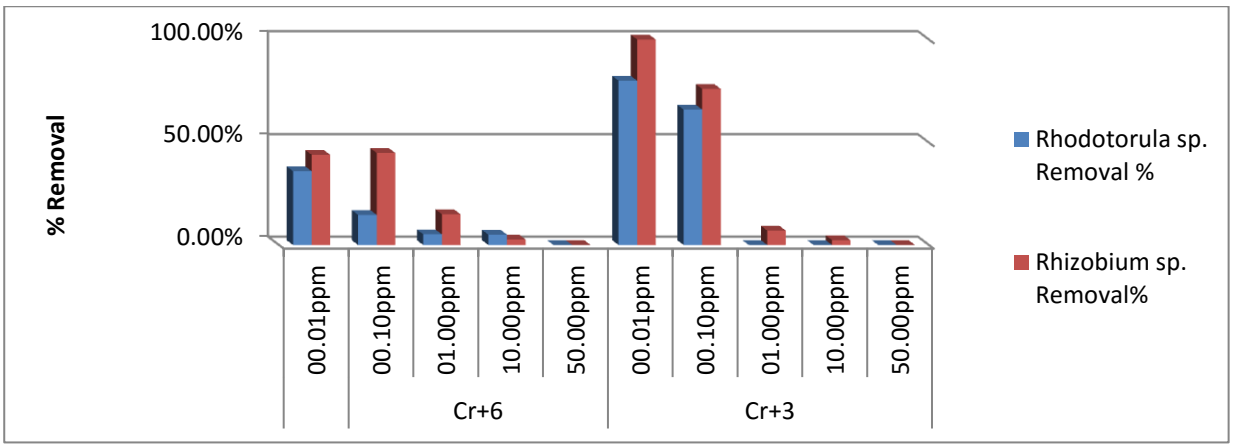

Fig. (4): Percentage of metal removal of Cr^{+3}& Cr^{+6} for both *Rhodotorula sp.*ALT72 and *Rhizobium sp.*MAP7-MG214656.

Table (1): Measuring remaining concentration with atomic absorption before and after culturing and percentage of metal removal of Cr^{+3}& Cr^{+6} for both *Rhodotorula sp.*ALT72 and *Rhizobium sp.*MAP7-MG214656.

Heavy metals conc		***Rhodotorula sp.***		***Rhizobium sp.***	
Type	**Control**	**Remaining**	**Removal %**	**Remaining**	**Removal%**
Cr^{+6}	00.01ppm	00.0064	36.000%	0.0056	44.000%
	00.10ppm	00.0853	14.700%	0.0551	44.900%
	01.00ppm	00.9480	05.200%	0.8497	15.030%
	10.00ppm	09.4930	05.070%	9.7296	02.704%
	50.00ppm	50.0000	00.000%	50.0000	00.000%
Cr^{+3}	00.01ppm	00.002	80.000%	0.000	100.00%
	00.10ppm	00.034	66.000%	0.024	76.00%
	01.00ppm	00.959	4.100%0	0.930	07.00%
	10.00ppm	9.353	6.470%0	9.764	02.36%
	50.00ppm	49.996	00.008%	50.00	00.00%

Discussion

Bioremoval of heavy metals has advantages like being highly selective, more efficient, easy to operate and hence cost-effective for treatment of large volumes of wastewater containing low metal ion concentrations[27]. Numerous bacteria are being researched upon for their ability to biosorb heavy metals [11]

It is clear from the results that *Rhodotorula*ALT72 and *Rhizobium*-MAP7-MG214656 can survive under stress of heavy metals ions (Cr^{+6} and Cr^{+3}), *Rhizobium*-MAP7-MG214656 showed higher ability of absorption/ adsorption potential than *Rhodotorula*ALT72 with concentrations 0.1 and 1 ppm which compared to the level of chromium permissible limit 0.05 ppm[28]. *Rhodotorula*ALT72on the other hand showed a better tolerance and uptake with Cr^{+3} ions. It was also reported that metal binding capacities of *Rhizobium*-MAP7-MG214656 was relatively higher than *Rhodotorula*ALT72. Different microbial sources like the green algae[29] and several fungi [30] are indeed good biosorbents like bacteria. It was reported earlier increased metal uptake by bacteria led to their death by various cell mediated mechanisms [31]. *Rhizobium*-MAP7-MG214656 gives higher ability of removal heavy metals and more resistive to metals ions than *Rhodotorula*ALT72. The two strains especially *Rhizobium*-MAP7-MG214656 were more effective in removal of heavy metals (Cr^{+6} and Cr^{+3}).

Conclusion

Microbial biomass is one of the most efficient biosorbents of heavy metals removal from solutions. Many researchers studied biosorption performance of different microbial biosorbents which provide application of biosorption technologies for heavy metal removal from solutions and also to understand the mechanism responsible for removal. It is expected to change in the near future, with

biosorption technology becoming more beneficial than currently used physicochemical technologies of heavy metal removal

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