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BIOTECHNOLOGY OF WASTE WATER TREATMENT WITH FUNGI (*ASPERGILLUS NIGER* AND *RHIZOPUS OLIGOSPORIUM*)

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Abstract

The present study deal with using biomass of two species of fungi, *Aspergillus niger*, *Rhizopus oligosporium*, in treatment of wastewater to reduce heavy metals concentrations (Pb, Cd) and some physical and chemical properties from final discharge of Al Nassirya city-Iraq .Tow group were used in this process, with tow weight from the mention fungi (1 ,2)gm of each group of fungi with 2 liter of wastewater this group have clone in laboratory in (25◦) extended for 6 days and register the results ever tow days .The result showed that high efficiency in reducing the concentration of (cd and pb) by fungi *Aspergillus niger* the percent of removing was 100% more than *Rhizopus oligosporium*, so that with reducing of some environmental parameters.

Keywords: Wastewater, Fungi and Heavy metals

Introduction

Direct discharge of wastewater is a major cause of contamination of the aquatic environment by many organic and inorganic pollutants, such as heavy metals, when applied to surface water without primary treatment. These pollutants change the properties of natural water. Heavy metals are dangerous pollutants to the environment (Korboule & Gilles 2007), there is environmental study of sewage direct discharge in Euphrates river and its effected on some physical, chemical and bacteriological parameters (Bushra *et al.*, 2013) in addition to polluted with dangerous bacteria (Bushra, 2016). Bioremediation has recently emerged as one of the most important alternative techniques in which microorganisms, fungi and plants are used to break down biodegradation and convert it to less toxic substances through metabolic processes or secretion of enzymes (Vidali, 2001). The aim of biodegradation is to reduce pollutants to acceptable and non-toxic levels (Pointing, 2001) or the transformation of organic pollutants into metals and carbon dioxide, becomes non-toxic substances (Gan & Koskinen, 1998). The biological breakdown of compounds occurs by multiple organisms and the process of bringing living organisms to the sites of pollution to increase the process of bioaugmentation (Norris *et al.*, 1993).

The most important aspect of biodegradation is to reduce the impact on the environment in order to become more suitable for human (Zhang & Chiao, 2002).

The entry of heavy metals into the water bodies reduces the balance of the ecosystem and the poisoning of aquatic plants, fish and other organisms in the environment. These minerals are very toxic to humans when they enter the food chain (Borgmann, 2005). Fungus plays an important role in reducing and removing the concentrations of many heavy metals in the soil and the aquatic environment for possessing a variety of different mechanisms that make them efficient in the process of reducing concentrations of heavy metals, such as the process of adsorption on the walls of the external fungal adsorption or the formation of heavy complexes sedated and storing elements within their cells (Guibal, 2001); Balderian, 2009). In their study, (Bennet & Fasion, (2008) noted that the fungal and dynamic fungi of the bioaccumulation of heavy metals and the ability of mycelium to efficiently absorb cadmium, iron, mercury and zinc in the aquatic environment. The fungal mycelium consists of a group of compounds such as polysaccharides and protein. The latter is composed of secondary groups such as carboxyl and hydroxyl, as well as contains phosphates and amino acids. All these groups of substances act on the binding of heavy element molecules (Gadd, 1992); (Veglio, and Beolchini, 2008).

The current study aims to compare the efficiency of the Mycelium fungus to the fungal biomass, *Aspergillus niger, Rhizopus oligosporium*, to reduce concentrations of heavy metals such as lead and cadmium and some physical and chemical properties of laboratory wastewater.

Materials and Methods

Sampling Method

Samples of the final discharge water of the sewage treatment plant in Nassirya were collected using 2liter polyethylene bottles, sealed and transferred to the laboratory, sterilized with autoclave, 121 bar, 15-bar.

Isolation, purification and diagnosis of fungi

Two types of fungi were isolated, developed and diagnosed from the sewage treatment plant in nassirya city, *Aspergillus Niger, Rhizopus oligosporium*, (Romero *et al.*, 2005). The fungal growth in malt extract agar (MEA) and added chloramphenicol was prepared by dissolving 250 mg of the antibody in 250 ml of distilled water. 1 mL of the sewage sample was placed in 9 cm diameter sterilized glass dishes. Add the sterilized stainless steel medium to the well, and incubate the dishes at 25 °C for 48 hours. The emergence of fungal colonies, worked slides for the purpose of diagnosis of fungal species and depending on the Classification (Webster, 1980).

Heavy metal measurement

Heavy metals were measured by lead and cadmium before and after treatment with fungus using an atomic absorption spectrophotometer. (APHA, 1998) was used for laboratory measurement. The percentage of removal of metal concentrations was calculated as follows:

$$\% = \text{Initial Focus - Final Focus / Initial Focus} \times 100\%$$

Chemical properties and physical properties

In determining chemical (COD) and physical properties include Total solids (TS)

Results and Discussion

Results of the study were shown in the table (1) the concentration of Lead after (2, 4, 6) days Treatment with (1) gm of *As. niger* are (10.01 Mg/L, 5.07 Mg/L, 0 Mg/L) while the concentration of Cadmium after (2, 4, 6) days Treatment with (1) gm *As. niger* are (0.17 Mg/L, 0.10 Mg/L, 0 Mg/L) in highly significant differences P<0.01.

The concentration of Lead after (2, 4, 6) days Treatment with (1) gm *R. oligosporium* are (12.11 Mg/l, 10.01 Mg/l, 6.43 Mg/l) and the Concentration of Cadmium after (2, 4, 6) days Treatment with (1) gm *R. oligosporium* are (0.21 Mg/l, 0.16 Mg/l, 0.09 Mg/l) in significant differences P<0.05, biomass of *Aspergillus niger* showed more ability to reduce Pb, Cd concentrations in 100% in 6 days period treatment.

Table 1 : Concentration of heavy metals before and after treatment with (1) gm of bio mass of fungi, *Aspergillus niger, Rhizopus oligosporium*

Element	Concentration before treatment (Mg / l)	Concentration after 2 days Treatment (Mg / l)		Concentration after 4 days Treatment (Mg / l)		Concentration after 6 days Treatment (Mg / l)	
		A. niger	*R. oligosporium*	*A. niger*	*R. oligosporium*	*A. niger*	*R. oligosporium*
Pb	14.5	10.01 **	12.11*	5.07**	10.01*	0	6.43*
Cd	0.25	0.17**	0.21*	0.10**	0.16*	0	0.09*

** Significant differences (P <0.01) compared to the treatment
*Significant differences (P <0.05) compared to the treatment

When used (2gm) of fungi biomass treatment (table 2) increase the percentage of removal of metal concentrations in short time.

Table 2 : Concentration of heavy metals before and after treatment with (2 gm) of biomass of fungi, *Aspergillus niger, Rhizopus oligosporium*

Element	Concentration before treatment (Mg / l)	Concentration after 2 days Treatment (Mg/l)		Concentration after 4 days Treatment (Mg/l)		Concentration after 6 days Treatment (Mg/l)	
		A. niger	*R. oligosporium*	*A. niger*	*R. oligosporium*	*A. niger*	*R. oligosporium*
Pb	14.5	5.77**	9.8**	0	6.03*	0	5.07*
Cd	0.25	0.09**	0.15*	0	0.11**	0	0.09*

** showed significant differences (P <0.01) compared to the treatment
* Significant differences (P <0.05) compared to the treatment

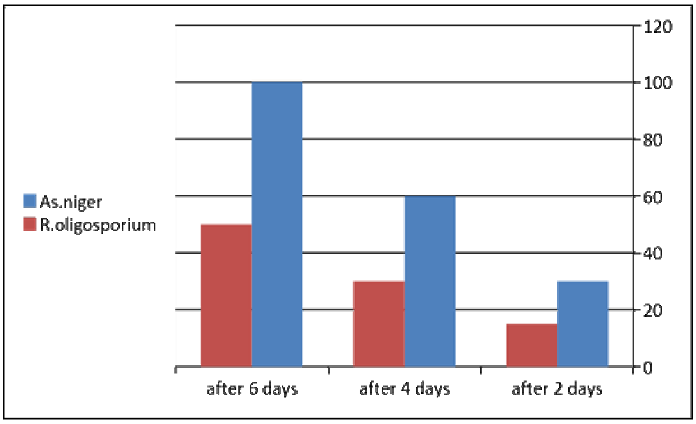

Fig. 1 : Removal ratio of lead when treated with (1) gm of the fungal biomass.

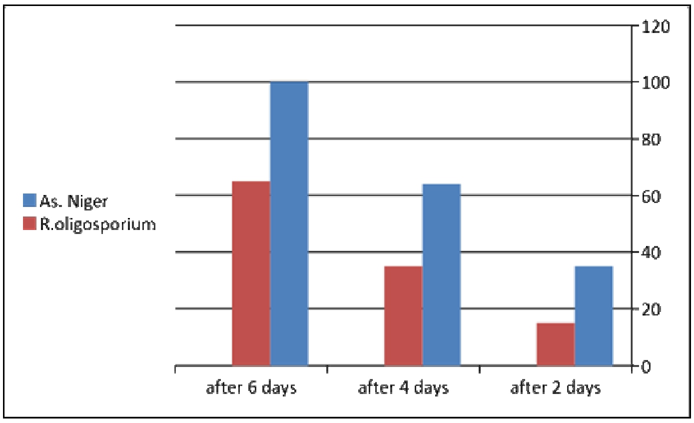

Fig. 3 : Removal ratio of cadmium element when treated with (1) gm of the fungal biomass.

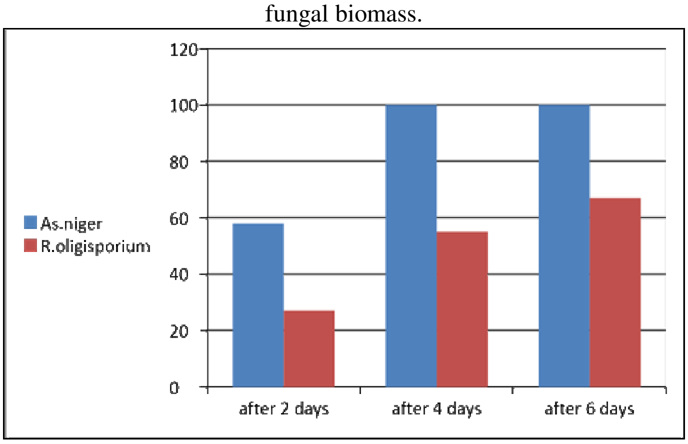

Fig. 2 : Removal ratio of lead when treated with (2) gm of the fungal biomass.

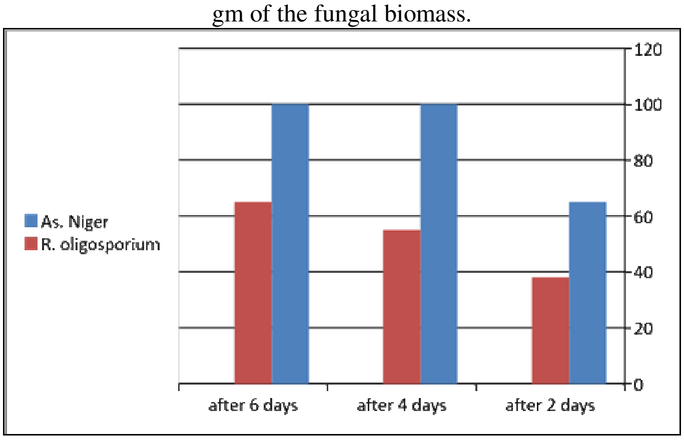

Fig. 4 : Removal ratio of cadmium when treated with (2) gm of the fungal biomass.

The results recorded the values of some physical and chemical properties that reduces when used (2 gm) of the biomass of the fungus, *Aspergillus niger, Rhizopus oligosporium* more than used(1 gm) of the biomass as showed in Table (3 ,4).

Table 3 : Values of some physical and chemical properties (mg / L) before and after treatment with (1 gm) of the biomass of the fungus, *Aspergillus niger, Rhizopus oligosporium.*

Properties	Concentration before treatment (Mg / l)	Concentration after 2 days Treatment (Mg / l)		Concentration after 4 days Treatment (Mg / l)		Concentration after 6 days Treatment (Mg / l)	
		A. niger	*R. oligosporium*	*A. niger*	*R. oligosporium*	*A. niger*	*R. oligosporium*
T.S.	171	150	167	125	145	88	102
COD	319	250	299	150	225	95	146

Table 4 : Values of some physical and chemical properties (mg / L) before and after treatment with (2 gm) of the biomass of the fungus, *Aspergillus niger, Rhizopus oligosporium.*

Properties	Concentration before treatment (Mg / l)	Concentration after 2 days Treatment (Mg / l)		Concentration after 4 days Treatment (Mg / l)		Concentration after 6 days Treatment (Mg / l)	
		A. niger	*R. oligosporium*	*A. niger*	*R. oligosporium*	*A. niger*	*R. oligosporium*
T.S.	171	120	144	78	97	45	54
COD	319	199	221	133	167	76	110

Table (1) shows the reduction of concentrations of some heavy metals lead and cadmium from the final discharge ponds of the wastewater treatment plant before and after treatment with one gram of live fungal mass. The results showed that the live mass of *Aspergillus niger is* highly efficient in removing concentrations of some heavy elements from wastewater P <0.01 higher than the efficiency of *R. oligosporium* within six days of treatment, and the higher the weight of the living fungal mass to 2 g the greater the efficiency of removal.

The results were consistent with the study of the efficiency of the fungal masses of *Aspergillus niger*, *Pencillium austurianum*, *Saccharomyces cervisiae* and *Mucor arcindloiddes* in reducing the concentration of heavy elements from contaminated soils (Omotayo *et al.*, 2008). They demonstrated the efficiency of these isolates in reducing iron concentrations by 60% The results were also consistent with (Iqbal, & Farah, 2009) in their study of the removal of chromium and cadmium from industrial waste water Using the non-living masses of *Aspergillus* and *Rhizopus* as they proved ability These fungi on the removal of these heavy elements within 18 hours of liquid farm, as well as (Teskova& Petrov, 2008) in their study of the fungus *Rhizopusdelemar* and the ability of its mass to reduce the concentrations of elements of liquid farm and efficiently. *Aspergillus niger* has been shown to reduce the concentrations of lead and cadmium elements by 100% during 6 days. This is due to the fact that its fungal yarn is cohesive and has a large mass of 973.4 mg, and direct contact with the wastewater sample has increased the surface area (Bai & Abraham, 2008) in their study on *Rhizopus nigricans* in removing the element of chromium from the liquid plant and the fungal spinning ability of this fungus to propagate significantly during the model and increase the surface area of the adsorption process. In his study, (Daniel *et al.,* 2006) observed the ability of the living mass of the fungus, *Armillaria*, to reduce concentrations of zinc, lead and cobalt from contaminated soils at high rates and for long periods of time through bioaccumulation (Preetha & Viruthagiri, 2009) also showed the ability of *Rhizopus* to reduce concentrations Zinc from the liquid farm is highly efficient. The results showed that the living mass of *Rhizopus* 543.2 mg was less efficient in reducing concentrations of lead, cadmium and copper due to the non-cohesion of the fungal yarn and the formation of a small thick mass, which led to weak contact with each sample of water as the fungus and its cohesion a large role and effective in the adsorption of heavy elements This was demonstrated by (Fourest, 2008) in their study comparing the efficiency of non-living masses of fungi *Rhizopus arrhizus, Mucor miechei, Penicillium chrysogenum* in reducing concentrations of heavy elements from contaminated soils.

The treatment recorded a decrease in the presence of suspended substances and in the waste water column as shown in Tables 3 and 4 due to bio-adsorption as well as bio-clotting. The volume decreased when biomass was used and the COD values decreased because the fungal cell membranes Functional groups that are negatively charged have the potential to adsorbs the elements and the positively charged minerals, then deposition and disposal, as well as the dismantling of complex materials into simpler materials that facilitate their exploitation by other organisms such as bacteria, fungi, yeasts, algae and primates.

Conclusions

1. The possibility of bio mass of fungus*, Aspergillus niger, Rhizopus oligosporium*, in reducing the concentration of heavy elements lead and cadmium at a high rate of 65-100% of the sample of sewage water.
2. *Aspergillus niger* showed the highest efficiency in reducing concentrations of heavy elements by 100% within 6 days treatment.
3. Showed the efficiency of *Rhizopus* less in the reduction of concentration of heavy elements lead and cadmium attributed to the fact that the mass is not coherent and thin.

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