THE POTENTIAL USE OF *Alternaria alternate* IN BIOREMEDIATION OF WASTEWATER CONTAMINATED BY HEXAVALENT CHROMIUM ION

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Abstract: Heavy metalpollution of the environment has become a global catastrophe that requires imminent attention. Several cleaning techniques including bioremediation with the use of bacteria and fungi have proven to be effective.In this study,the potential of *Alternaria alternata* isolated from sorghum grain in bioremediation of waste water contaminated with hexavalent chromium ion was assayed by determining its chromium uptake, mycelia growth, glucose uptake and effect of pH in medium with different concentrations of potassium heptadichromate (VII) ion ($K_2Cr_2O_7$). The maximum average mycelia growth of 2.48 cm was achieved at 0.05 g/100mL concentration of $K_2Cr_2O_7$, however, growth was inhibited at higher concentrations of 0.25 g/100mL as indicated by a decrease in average mycelia growth to 1.48cm. A decrease in glucose concentration was also observed as $K_2Cr_2O_7$ concentration decreasedwith maximum decreaseobserved at 0.05 g/100mL after 9 days. The pH of the media containing varying concentrations of $K_2Cr_2O_7$ (0.05-0.25 g/100mL) changed from basic to acidic as the organism grow. At low concentration of 0.05 g/100mL of $K_2Cr_2O_7$, media pH changed from 9.09-5.03 and 8.60-6.65 at high concentration of 0.25g. *Alternaria alternata* species have shown to be important fungi with promising potentials of bioremediating wastewatercontaminated with hexavalent chromium ion.

Keywords: *Alternaria alternate*, bioremediation, hexavalent chromium, wastewater.

Introduction

The continuous effort by man to ensure his environment is eco-friendly, has ledto an unwavering search for new technologies that will be vital in eliminating various pollutants from the environment. Heavy metals pollution of the environment has become a global catastrophe that requires imminent attention. Environmental pollution with metals, semi-metals and organic contaminants is a serious global problem, with heavy metals being one of the most dangerous pollutants(Xiezhi *et al.*, 2005). Occurrence of heavy metals in our environment causes adverse effects on flora, fauna and groundwater contamination through leaching. Heavy metal contamination alsoresults to reduced performance and product quality in agriculture and is dangerous for public health and other living organisms (Khosravi *et al.*, 2009). Their existence in soil result to environmental stresses that can lead to reduction of plant growth (Khosravi *et al*., 2009; Mohsenzade *et al*., 2012).

Among the major source of heavy metals release into the environment is sewage and effluents from various industrial activities such as metal plating, paint industry, metallurgy, released oil ingredients in the soil, combustion of fossil fuels, mining, ores washing, pesticides, colored material, batteries, natural erosion of rocks and so on (Vadkertiova and Slavikova, 2006). Due to their non-biodegradable, toxicity and bioaccumulative nature, these compounds particularly interfere with cellular and enzymatic processes, thereby modifying them in ways that can cause severe detrimental effects on such systems as; gastrointestinal, respiratory and nervous organs and tend to cause cell necrosis in the exposed organs (Saberi *et al*., 2010). When heavy metals accumulate in the soil, they affectmicrobial activity and can also put human health at risk as they become part of the food chain. Toxic effects of metals are also experienced in various processes such as reduction of nitrogen fixation in plants, irregularities in synthesis of enzymes (Saberi *et al*., 2010). Trivalent chromium (Cr^{3+}) in trace amounts has influencein sugar and lipidmetabolism in humansand its deficiency is suspected to cause a disease called chromium deficiency. In contrast, hexavalent chromium (Cr^{6+}) is very toxic and mutagenic when inhaled (Salnikow and Zhitkovich, 2008). Several conventional treatment technologies, such as chemical precipitation, ion exchange, membrane technologies, electroplating, adsorption etc. have been found to be very expensive and difficult to maintain due to high capital and operational costs (Marin-Rangel *et al*., 2012; Mishra *et al*., 2012; Banerjee *et al*., 2012). There is also an extra cost of treating the resultant sludge and secondary waste generated, before disposal as it also poses hazards and pollution risks to the environment (Kumar, 2006). Due to these challenges associated with the conventional methods, there has been growing interest and research into the use of microorganism for remediation. It is notable that somemicroorganisms use these contaminants as a source of nutrients and energy and convert them into soluble substances, in a process known as bioremediation (Kumar *et al.*, 2008). This method has shown promising possibility of removing one or moreof these pollutants from the environment, with high efficiency, low cost, and release of end products with less detrimental effects on the ecosystem of contaminated sites (Mohsenzade *et al.*, 2012). The use of microorganism such as algae, fungi, bacteria, and yeasts that can absorb heavy metals, have been considered by some prior researchers for bioremediation of heavy metal polluted media (Xiezhi *et al.*, 2005; Pradhan*et al.*, 2007; Svecova *et al.*, 2006). These microorganisms immobilise metal ions by linking them with their cell walls (Akhtar *et al*., 1996; Vankar and Bajpai, 2008).

Mycoremediation is a form of bioremediation that involves using fungi to degrade or sequester contaminants in the environment. The choice of biomass for use in bioremediation is of utmost importance.Hence it should be abundant in the environment and able to be adapted to

varying environmental conditions (Mohsenzadeh *et al.,* 2010; Kumar *et al.,* 2012). Many contaminants can be reduced by means of biological methods and some of them can be remediated by fungi (Dugal and Gangawane, 2012). Some fungi are hyperaccumulators, capable of absorbing and concentrating heavy metals in the mushroom fruit bodies (Singh, 2006). One of the primary roles of fungi in the ecosystem is decomposition, which is performed by the mycelium that can reduce toxins in-situ.

Some researchers were able to remove Cd, Ni and Pbbiologically using fungi with the efficiency of 94.47% for Cd, 79.81% for Ni and 99.73% for Pb (Farzin, 2010). In another research, chromium and nickel uptake by resistant bacteria and fungi was studied and results showed that removal efficiency of nickel was 90% with the amount of 0.1 milligrams of fungal biomass of *Aspergillusniger* (Amini *et al.,* 2008). Also in other study, 0.7 g/L of fungal biomass of *Aspergillusniger* showed 84% removal of cadmium ions (Congeevaram *et al.,* 2007; Barros *et al.,* 2003). *Alternaria* is a genus of ascomycete fungi (Jingfeng*et al*., 2013). *Alternaria* species are known as major plant pathogens. They are also common allergens in humans, growing indoors and causing hay fever or hypersensitivity reactions that sometimes lead to asthma (Jingfeng *et al*., 2013). They are ubiquitous in the environment and are a natural part of fungal flora almost everywhere. They are normal agents of decay and decomposition. The spores are airborne and found in the soil and water, as well as indoors and on objects. Not all *Alternaria* species are pests and pathogens; some have shown promise as biocontrolactivities against invasive plant species (Meagher, 200). The aim of thisstudy is to ascertain the potential use of *Alternaria alternate* in Bioremediation of Wastewater Contaminated with Hexavalent Chromium ion.

Materials and Methods

Source of materials

Sorghum grain samples were collected from Maiduguri metropolitan area in Borno State, and identified at the Department of Botany University of Maiduguri. The identified samples were then taken to the biotechnology LaboratoryUniversity of Maiduguriforprocessing to isolate fungi species (*Alternariaalternata*) required for this research work. All reagents used were of analytical grade.

Isolation ofAlternariaalternata

Plant samples contaminated by *Alternaria alternate* fungi were inoculated into sterile potato dextrose agar (PDA) using sterilized forceps into the culture plates at room temperature to observe growth of the fungi. On establishment of growth after 48h of incubation, the growth was subcultured in a fresh sterile PDA and allowed at room temperature for five days for purity.

Identification of fungi

The isolated fungus was identified based on their colony characteristics as well as their vegetative and reproductive structures as observed under the electronic microscope. Some macroscopic and microscopic characteristics considered include; color of the colony, shape of the conidia head, pattern of arrangement of spores on the conidia. Identification was done using standard manual (Feng and Ma, 2010).

Preparation of treatment

The treatments were prepared according to the method described by Dawodu and Okpomie (2014). Exactly 0.05 g, 0.10 g, 0.15 g, 0.20 g and 0.25 g) of potassium dichromate were weighed and separately dissolved in 100mLof distilled water in 250mLcornical flask. Exactly 200 µl each of the prepared treatment was added to molten potatoes dextrose agar (PDA) (45^{o}C) and mixed. Thesewere distributed in 20 mL aliquots in sterile petri dishes and allowed to solidify. The isolated fungus of choice was then inoculated into the different Petridishes with inoculating needle to observe the mycelia growth of the fungus in different concentrations of the treatment.

Preparation of liquid treatment

Modified Vogel's mineral salts medium containing 10 g glucose, 1.65 g $(NH_4)_2SO_4$, 0.67 g NH_4Cl, 0.1 g $MgSO_4.7H_2O$, 2.5 g KH_2PO_4, 1.5 g $NaH_2PO_4.2H_2O$, 0.03 g Na_2SO_4, 0.08 g K_2SO_4, and 0.1 g $MgCl_2$ per liters were used. Exactly 200 µl each of the different concentrations of potassium dichromate was dissolved in 100 mL of liquid medium.This was inoculated with *Alternaria alternate* for one day (Mohsenzadeh *et al*., 2014).

Determination of growth/chromium uptake

The tolerance of the isolated *Alternariaalternata* to chromium was studied by growing the organism on culture plates containing varying concentrations of potassium hexavalent chromium (VII) ionas prepared above.The plate without potassium dichromate served as control. Diagonal lines were initially ruled at the back of the agar plates using a bold marker to ease measurements of mycelia diameter. The culture plates were incubated at 30°C for twelve days. Mycelia diameters were measured in centimeters from the first to the twelfth (1^{st} – 12^{th}) day, and the average growth for various concentrations including the control growth was recorded (Tran *et al.,* 2011).

Determination of glucose uptake byAlternariaalternata

Glucose concentration was determined after enzymatic oxidation with glucose oxidase reagent (Randox laboratories L.T.D. UK). Exactly 200 µl of the glucose reagent was reacted with 5 ml of the treated liquid medium containing the fungus and various concentrations of potassium dichromate. The samples were mixed well and incubated for 10min at 37^{o}C. The absorbance of the samples (A sample) and standard (A standard) were measured against a reagent blank within 60 min. Samples were read three times each after 72 h and tabulated.

Glucose concentration was calculated as follows:

Glucose concentration (mg/dl) = A sample/A standard x Concentration of standard

Glucose Concentration (Mmol/l) = A sample/A standard x Concentration of standard x 5.5.

Glucose standard = 1.182 mg/dl.

The glucose concentration was varied with varying concentrations of $K_2Cr_2O_7$ as 38.22 mg/dl, 27.18 mg/dl, 22.40 mg/dl, 18.82 mg/dl and 15.42 mg/dl, respectively.

pH mesurement

Alternariaalternata was inoculated into the liquid medium containing various treatments. The pH of each treatment was measured in triplicate each after 72 h using the pH meter; the control pH 0f control was also recorded.

Results and Discussion

Potential use of Alternariaalternatain bioremediation.

Fig. 1 presents the average mycelia diameter of *Alternariaalternata* in various treatment of potassium dichromate($K_2Cr_2O_7$), a hexavalent metal. There was decrease in the mycelia growth as the concentration of the hexavalent metal increased. Ranging from 1.47 cm in control to 2.48 cm in sample treated with 0.05g of $K_2Cr_2O_7$.The effect of hexavalent chromium on the glucose uptake by *Alternariaalternata* shows a decrease in glucose concentration as the incubation days increases (Fig. 2). At 0.05 g chromium concentration, the glucose concentration decreased from 38.22 mg/dl to 9.30 mg/dl. It decreased from 27.18 mg/dl to 14.07 mg/dl, from 22.40 mg/dl to 13.39 mg/dl, from 18.82 mg/dl to 9.16 mg/dl and 15.42 mg/dl to 8.54 mg/dl for chromium concentrations of 0.10 g, 0.15 g, 0.20 g, and 0.25 g, respectively.The effect of the growth of *Alternaria alternata* on the pH of the medium treated with potassium dichromate ($K_2Cr_2O_7$) is presented in Fig. 3. The result showed a gradual decrease in pH toward acidity with increasing time of incubation.

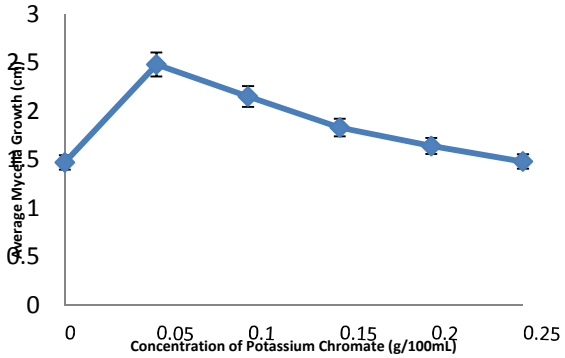

Fig. 1: The average mycelia growth of *Alternaria alternata* in various treatment and control

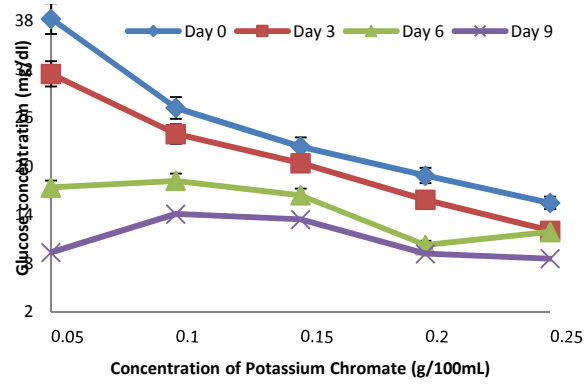

Fig. 2: Residual glucose concentrations (mg/dl) in *Alternariaalternata* culture treated with hexavelent metal.

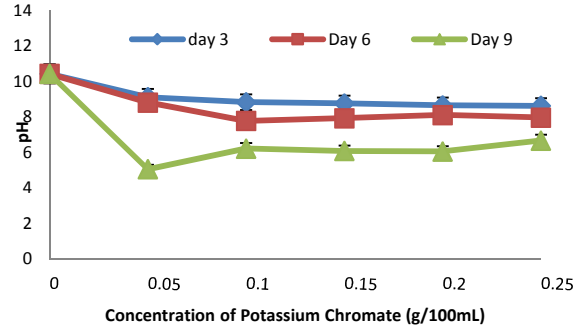

Fig. 3: Changes in pH with growth time in *Alternariaalternata* culture treated with hexavelent chromium.

In this present study, the average mycelia diameter of the fungus in various treatments show higher growth compared with the control.This indicates that the organism is utilizing chromium for its growth (Kaiser *et al*., 2008). At chromium concentration of 0.05 g, the fungus show maximum mycelia growth of 2.48 cm, but as the concentration of potassium dichromate increases, the growth decreases at 0.25 g, with average mycelia diameter of 1.48 cm. The observed decrease in growth rates with increasing concentrations of chromium treatment indicates that chromate is inhibitory to the growth of the organism at higher concentrations.Chromates are known to inhibit the growth of most organisms, although resistance to the toxic metal may be developed by the selection of resistant variants to cope with such toxicities (Olukoya *et al*., 1997).

It was observed that the glucose concentrations decreased with increasing time of incubation and growth of *Alternariaalternate* in the present study. This signifies that the fungus was utilizing the glucose for its mycelia growth and metabolism. At 0.05 g/100mL concentration of potassium dichromate it was noticed that glucose uptake was at maximum, the glucose concentration was 31.38 mg/dl after 72 h and it decreases rapidly to 17.34 mg/dl at day 6 and then 9.30 mg/dl after 9 days. At 0.25 g/100mL concentrations, the glucose uptake was low, the glucose concentration decreased from 12.01 mg/dl at day 3 to 8.54 mg/dl at day 9. It can be inferred that glucose uptake is higher at lower concentration of potassium dichromate than at higher concentration of chromium possibly due to the inhibition of utilization of glucose and metabolic activities and hence growth of the fungus as observed by Kaiser *et al*. (2008) on their studies on *In-vitro* inhibition of mycelial growth of several phytopathogenic fungi, including *Phytophthoracinnamomi* by soluble silicon.

In the present study pH generally varies in all the concentration of chromium from alkalinity to acidity with incubation time. pH is directly proportional to the growth of the organism, the release of metabolites by the organism i.e. release of carbon dioxide (CO_2) by fungus which combines with water (H_2O) to form carbonic acid (H_2CO_3)and this makes the medium acidic. The growth of most filamentous fungi have been reported to be optimal under acidic conditions particularly at pH values between 3 and 4.5; also for some filamentous fungi such as trichoderma, penicilium, etc., the optimal pH for maximum activity lies between 3 and 4 (Mohsenzadeh and Shahrokhi, 2014). Since pH is directly proportional to growth, it indicates that acidic pH supports the fungal growth.

Conclusion

From the results obtained in this study, it has been observed that high concentrations of hexavalent chromium ion inhibit the growth of *Alternaria alternate.* pH of the media was an important factor that affected the growth of the organism, growth was more favourable in an acidic media. *Alternaria alternate* has shown promising potential for biodegradinghexavelent metal (potassium dichromate) and thus this technology may be explored in the bioremediation of environments contaminated by this heavy metal.

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