Acclimatization Potential Isolated Fungi from Tannery Waste and Land Fill to Various Chrome Concentrations

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ABSTRCT

In determining the tolerance of the fungi isolated and characterized from tannery effluent and landfill to various concentrations of chrome in the laboratory, a relatively higher tolerance to chrome solution than the previously studied bacteria species was observed. Four fungi: *Aspergillus Niger, Rhizopus nigricans,_Aspergillus flavus and Aspergillus fumigatus* – were tolerant in varying degrees to chrome solutions up to 3.0% and dying from 3.5% at an optimum P^H and temperature of 4.5 and 37^0 C respectively in 4 days. Fungi from control soil environments other than the tannery landfill did not tolerate beyond 1.0% chrome solution, growing maximally between 4 and 26 days. The hope of using cheaper viable fungal biomass to remediate tannery waste and landfill environments as substitution for the costly physicochemical methods is kindled.

Keyword: Chromium. Fungi, Bioremediation, Tanney Effluent and Landfill

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INTRODUCTION

Heavy metals are present in high concentrations in a wide variety of industrial effluents such as textile, leather, tannery, electroplating, galvanizing, pigment and dyes, metallurgical and paint industries (Zhang et al, 2005; Ahluwalia and Goyal, 2007 and Maszenan et al., 2011).

In recent years, biosorption of heavy metals by microbial cells has been recognized as a potential alternative to the existing technologies for removing heavy metals from industrial effluent (Vasu vevan et al., 2001; Tobin and Roux, 2008; Huang et al., 2010; Jinger and Saliu, 2014), and accumulation of heavy metals by microorganisms or their products has received more attention as the metal ion concentration less than $10mgl^{-1}$ can thus be removed (Saswati et al., 1998). These bioremedial processes use either viable or non – viable cells. Bacteria, algae, yeasts and other fungi have been used successfully as adsorbing agents for heavy metals (Kumar et al., 1998).

There are about 65 elements which possess metallic properties, have a specific gravity greater than five and may, therefore, be considered as heavy metals. Most of these are either insoluble or extremely rare and their effects on biological systems are of minor importance. However, some of them are potentially able to interact with microorganisms. These are essential for growth, reproduction and /or, survival, while others have no known biological function and are toxic. The demarcation between essentiality and toxicity is not distinct and usually depends upon the active concentration of a heavy metal that is accessible to biological systems (Daxbury, 1986). Consequently, several heavy metals required in trace amounts for variety of environmental processes may produce deleterious effects when they are present as pollutants.

Microorganisms have thus been found to influence the behaviour of inorganic compound in ways that are of potential use in bioremediation (Erlich and Brirely, 1990). These include the concentration of metals or redionucleids in the environment by modification of physico – chemical condition and destruction of other inorganics such as nitrates or chromates that are considered toxic or hazardous at high concentrations or under specific circumstances.

The ultimate aim of this research effort is the removal of chromium, a heavy metal, from tannery effluent, using the adsorptive abilities of either living or dead fungal mycelium. Such biological approach to metal ion recovery can be used to clean up effluents or to recover precious metal ions from solution. In both cases, it will be necessary to show that the use of fungal biomass can compete with physico-chemical methods that has yet to be conclusively demonstrated.

Tannery waste mainly contains trivalent chromium. (Cr III), out of the two stable states of chromium, Cr^{3t} and Cr6t (Mason etal., 1990; Alexander et al; 1992; Menden and Rutland,1998). However, the regulations about the disposal of such wastes are based on the probable presence of hexavalent chromium (through oxidation), which is considered to be toxic, carcinogenic, teratogenic and mutatgenic in animals,, hence requiring special attention (Roe and Certer,1969). However; the regulations about the disposal of such wastes are based on the probable presence of hexavalent chromium (through oxidation), which is considered to be toxic, carcinogenic, teratogenic and mutagenic in animals, hence requiring special attention (Roe and Carter, 1969).

Preliminary investigation to this study examined the types of microorganisms existing in a case tannery in Kano, Nigeria with the aim of knowing the ones seemingly tolerant to the obviously toxic environment, which may be used in removing chrome from the environment of final dumping – the landfill (Okonkwo et al, 2001). Gram – negative rods and Gram-positive cocci were the bacteria found, while *Aspergillus sp.* were the fungi observed. The laboratory acclimatization of the bacteria to chrome (Okonkwo et al, 2003) had them tolerant to 0.1% chrome Concentration; dying in subsequent concentrations. The identification and characterization of the fungi in the tannery's landfill soil as well as nearby control soils revealed four fungi – *A.* fungatus; *A.flavus*; A. *niger and* R. *nigricans* were in the landfill as well as on a tomato farmland and an unfarmed control site (Gbolagunte et al., 2003). The control sites had *Paracoccoid braisilensis* and *Candida albicans.* The *tomato farmland had Cephalosporum sp., Penicillium expansium* and *Trichophyton scholeniin* in addition to the three *Aspergillus*p and one *Rhizopus sp.* of the landfill.

All these fungi are intended for similar laboratory acchimatization to various concentrations of chromium – as earlier done for bacteria (Okonkwo et al., 2003) – in this study. The aim is to know which fungi are tolerant and to what extent are they, to various concentrations of absolute chrome solution. Thereafter, adsorptive experiments will be carried out in order to achieve the intended bioremediation of the landfill soil.

MATERIALS AND METHODS

Different concentrations of chrome were added to the pure isolates of the fungi from the cultures from various soils from which samples were previously collected (Gbolagunte et al., 2003). 0.1 ml of each soil culture was inoculated into different petridisges containing the various media (Saboraud Dextrose Agar (SDA); Extra Malt Agar (EMA) and potato Dextrose Agar (PDA), and were incubated at 37^0c for 4,7,9,21, and 26 days respectively. The three media were for fungal isolation. Initially, three concentrations of chrome were used from 0.1% to 1% as was done for bacteria (Okonkwo et.al 2003) but with observation of growth in some of them, it was increased to eight concentrations- 0.1, 0.5, 1.0, 1.5, 2.0, 2.50, 3.0, 3.50, percent respectively. Optimum pH and temperature for growth were observed for the various isolated fungi, the number of days for maximum growth was noted.

RESULTS

The acclimatization pattern in the various chrome concentrations were as shown on table 1, where Aspergillus niger had the most abundant growth and still had moderate growth in 3.0% chrome concentration, finally dying in 3.5% chrome solution. This was followed by *Rhizopus nigricans* which had scanty growth in 3.0%, and then *A. Flavus* had moderate growth in 1.0% and scanty growth at a pH of between 4 and 5 and at an optimum temperature of 37^0c in 4 days. The other remaining fungi did not do too well in chrome except *Penicillium expansium* which followed *A. Fumigatus* closely – having only scanty growth in 0.5 and 1.0 percent respectively. Its maximum growth took 7 days. *Coccidiode immitis,* which grew only abundantly in an environment devoid of chrome, did so in 21days, at an optimum pH between 5 and 6 (Table 1).

TABLE I-ACCLIMATIZATIN PATTERN OF THE CHARACTERIZED AND ISOLATED FUNGI FROM CHROME TANNERY EFFLUENT LANDFILL AND NEARBY SOILS IN CHROME SOLUTION

Type of Organisms	Concentration									Optimum pH	Optimum temperature	Day of Maximum growth
	0.00	0.10	0.50	1.0	1.50	2.0	2.50	3.0	3.50			
Aspergillus niger	++++	++++	++++	++++	+++	+++	++	++	-	4 - 5	37^0c	4
Rhizopus nigricans	++++	++++	++++	++++	+++	+++	+	+	-	4 – 5	37^0c	4
Aspergillus flavus	++++	++++	+++	++	+	-	-	-	-	4 – 5	37^0c	4
Aspergillus fumigates	++++	++++	+++	+	+	-	-	-	-	4 – 5	37^0c	4
Candida albicans	++++	++	-	-	-	-	-	-	-	3 – 4	28 – 30^0c	9
Pencillium notatum	++++	+++	+	-	-	-	-	-	-	4 – 5	37^0c	4
Paracoccoid brasilensis	++++	+	-	-	-	-	-	-	-	5 – 6	37^0c	26
Cephalosporuim sp.	+++	-	-	-	-	-	-	-	-	5 – 6	37^0c	9
Geotrichum sp.	+++	+	-	-	-	-	-	-	-	3 – 4	37^0c	9
Pencillium expansium	+++	+++	+	+	-	-	-	-	-	4 – 5	37^0c	7
Coccidiode immitis	+++	-	-	-	-	-	-	-	-	5 - 6	37^0c	21

++++	Most Abundant
+++	Abundant
++	Moderate
+	Scanty
-	None

DISCUSSION

The fungi types from the landfills (Gbolagunte et al., 2003) which also existed in the tannery effluent (Okonkwo et al, 2001), acclimatized better to chrome environment in this study than did the bacteria of tannery effluent (Okonkwo et al, 2003). The bacteria, only survived up to 0.1%, whereas, the fungi – *A. Niger*, *R. nigricans*, A. Flavus *and A. Fumigatus* in this study, survived variously from 1.50 to 3.0 percent chrome environment.

Since it is obvious that fungi may have adaptive advantage to chrome environment than bacteria, our bioremediation effort may focus more on the use of these favorable fungi. An area of fungi biotechnology currently in vogue is the use of fungal biomass to adsorb metal ions from solution (Gadd, 1990; Zhang et al, 2005) Yao et.al, 2009 and Maszenan et al, 2011; Huang and Huang, 2016; Huang et al, 2010; Jigger and Sahu, 2014; Farhan and Khadom, 2015). The intention is the removal of pollutant heavy metal or radionucleides from effluents and landfill soils using adsorptive abilities of either living or dead fungal mycelium. Such biological approach to metal ion recovery can be used to clean up polluted effluents or to recover precious metals ions from solution, in such a case, it will be necessary to show that the use of fungal biomass can compete favorably with physicochemical methods.

A variety of technologies are currently available to treat soils contaminated with hazardous materials, including excavation and burial in a chemical secure landfill, vapour extraction, stabilization and solidification, soil washing, soil flushing, critical fluid extraction, chemical precipitation, verification, thermal desorption and incineration (U.S.EPA, 1988). Many of these physicochemical treatment technologies do not actually destroy the hazardous compounds present. Rather, the chemicals may simply be bound in a modified matrix or transferred from one phase or location to another. Because these methods do not destroy the contamination and because they are often costly, there is a strong incenive to develop and apply innovative above ground (ex-sittu) and in place (in-sittu) remediation methods.

Bioremediation, the utilization of microorganisms to metabolically mediate desired chemical reactions or physical processes, can often fill this need or contribute to the solution (Grady, 1985, Thomas and Ward, 1989).

The adsorption of the metals and microbial biomass can however, also prevent further migration of contaminants. Biosorption is the process by which metals are sorbed and or complexed to either living or dead biomass (Derek and John, 1997). Several factors that influence the microbal removal of heavy metals from aqueous solutions include the specific surface properties of organism, the cell metabolism and the physicochemical parameters of the environment (Suresh Kumar et al, 1998). Different chemicals on the biomass cell walls such as chitin, aminoacid carboxyl groups etcetera. could provide binding sites or heavy metal ions (Sudha bai and Emilia Abraham, 1998). Furthermore, the biomass polysaccharides exhibits ion exchange properties (Kuyucak and Volesky, 1989; Treen Sears et al., 1984).

The issue in this study is that since these fungi were tolerant of the chrome environment to the extent that they were, their possible viability in the land fill is highly likely. Therefore, if made to grow abundantly (with the aid of suitable substrates) in the land fill, the tendency of extensive adsorption of the of the choromium to their surfaces is high, since fungal surface contains metal binding ligands such as chitin, amino group, sulfurhydryl group etcetera. This can prevent futher migration of contaminants. The process may not only be that of sorption, it may infact be complexing - either way, the union becomes non-reactive or inert, thereby allaying the fears of where to desorp the supposedly toxic absorbed metal. Microorganisms can remove toxic metals and metalloids from contaminated water and waste stream by converting them into forms that are precipitated or volatilized from solution (Suresh Kumar et al., 1998). Accumulation of metals or their products by micro-organisms has been used for some -time, but has received more attention in recent years because of its potential application in both environmental protection and recovery of precious and stragetic metals (Gadd and Rome, 1988)

Reason for varying the concentrations of the chromium solution in these study is that of toxicity which is well known to be limiting. Microbial growth actually, will be influenced by the presence or absence of toxic or inhibitory materials (Skladamy and Metting Jr.,1993). It is important to remember that inhibition or toxicity is often a result of high contaminant concentrations and not merely due to their presence. Certain chemicals may only inhibit the growth of a given species, whereas other compounds may actually be lethal at the same concentration .For biotreatment to be successful, concentration of toxic chemicals must be carefully evaluated even when they are the target contaminants. That is what this study has addressed.

Successful soil bioremediation relies on identifying and maintaining a suitable P^H for microbial biodegradation of the contaminants of interest (Skladamy and Metting, Jr., 1993). The four fungi of interest in this study grew optimally at P^H 4 to5 at 37^oc in four days. Soil and ground water remediation occur, at a P^H of 5 or less (Skladamy and Metting, Jr., 1993; Zhang et al., 2015; Yao et al., 2009) because at a P^H greater than 5, precipitation of $Cr(OH)_3$ occurs and competes with functional groups of biosorption which are caboxyl, amine and phosphate groups. At P^H 5 or less, optimum environment is provided for the growth of their fungi, if the process would require that the biomass should be viable.

CONCLUSION

Two fungi – *Aspergillus niger* and *Rhizopus nigricans* – out of the four found in tannery waste and landfill, tolerated chrome solution of up to 3.0% concentrations, while the other two – *Aspergillus flavus* and *Aspergillus fumigatus.* – only tolerated up to 1.5%; all at an optimum P^H and temperature of 4 to 5 and 37^0C respectively in four (4) days. The fungi found in the control lands had only *Penicillium expansium* tolerating up to 1.0% chrome solution. The others tolerated less at 4 -6 P^H.and 28 – 37^0C in 4 to 26 days. The relatively higher tolerance of the four tannery waste/ landfill fungi opens up their favourable potential for cell surface adsorption of the chromium metal, away from the environment, substituting this cheaper and the cleaner biological remediating method for the costlier and not –too-environment friendly physiocochemical methods.

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