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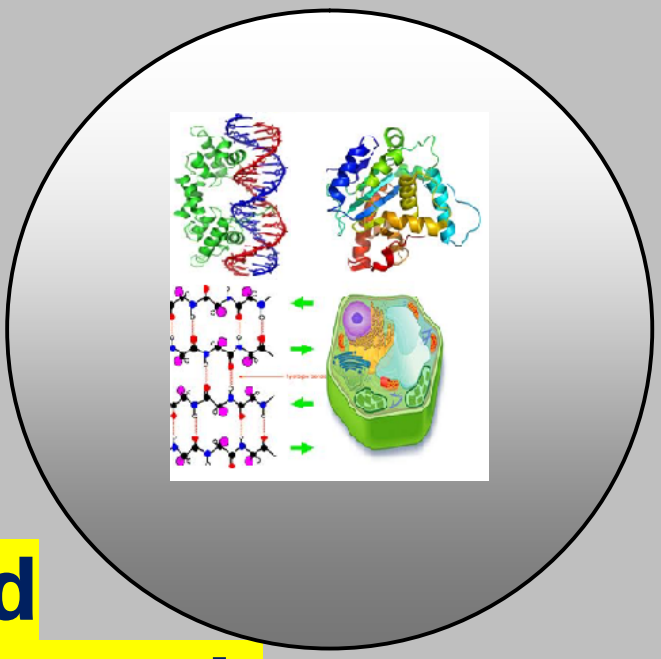

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RESEARCH PAPER

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Bioremediation of Match Industry waste by fungal Isolates

K. Parani, R. Rani and P. Selvarathi

Department of Botany with Specialization in Plant Biotechnology,
The Standard Fireworks Rajaratnam College for Women (Autonomous),
Sivakasi, Tamilnadu, India

ABSTRACT

In the present study, the match industry waste soil is brown in colour with chlorate odour. The dark brown colour of the match industry waste soil is due to the presence of raw material used. The Electrical Conductivity (EC) of the match industry waste soil is very high. Fungal isolate M_1 and M_2 were highly resistant to toxic elements present in match industry waste soil were followed by M_3, M_4, M_5 in decreasing order respectively. Out of the 5 fungi isolated, only 2 fungal species such as Aspergillus terreus, A. flavus showed the most prominent growth on medium with 10 %(w /w) matches' industry waste soil.

Keywords: Fungal isolates, Match industry waste soil, Electrical Conductivity, Aspergillus species and Bioremediation.

INTRODUCTION

Bioremediation is the use of living organism to degrade environmental pollutants or to prevent pollution through waste treatment. Bioremediation is an emerging and the most ideal alternative technology for removing pollutants from the environment, restoring contaminated sites and preventing further pollution. This environment friendly technology is expanding with range of organisms to be used to clean up pollution and forms vital component of the green movement of maintaining the nature's overall ecological balance an issue at present occupies top priority of environmental awareness and public policy (Yang and Illman, 1999).

Due to urbanization and land degradation, the area of agricultural land is continuously decreasing. Proficient use of available agricultural land resources is important to overcome the deficiency. Among the soil quality maintenance the heavy metals plays an imperative role to sustain its eminent properties (Mc Grath *et al.,* 1995; Cheng, 2003). The possibility of using bioremediation to restore environments contaminated with organics is well known. Bioremediation may either be intrinsic bioremediation which naturally takes place in contaminated environments or engineered. The availability of variety of microbial biomass and their metal binding potential makes it economical and sustainable option for developing effluent treatment process for removal and recovery of heavy metals. Metals are toxic to all biological systems from microbes to plant and animal, but microorganisms affected more than other systems, due to their small size and direct involvement with their environment (Patel *et al*., 2007; Sarret *et al.,* 2005; Giller *et al.,*1999). Metal toxicity negatively impacts all cellular processes, influencing, metabolism genetic fidelity and growth loss of bacterial population in metal - contaminated soils, impacts elemental cycling, organic remediation efforts, plant growth and soil structure.

Most of the studies dealing with microbial metal remediation via growing cells describe the biphasic uptake of metals that is initial rapid phase of biosorption followed by slower, metabolism dependent active uptake of metals (Garnham *et al.,* 1992, Donmez and Aksu, 1999). Numerous fungi can persist in a polluted milieu either in a developing state or as dormant forms such as chlamydospores of mucorales. The species able to resist and grow are naturally selected in contaminated soils and often valuable for a bioremediation purpose (Chafai, 1996, Bordjiba *et al.,* 2001). Soil is a non-renewable dynamic resource and acts as an interface between agriculture and the environment. Maintaining soil quality is the vital factor to improve crop yield and productivity. In this light, biological materials have emerged as an eco-friendly and economical option (Ting *et al.*, 1988.). Biological processes rely on useful microbial reactions including degradation and detoxification of hazardous organics, inorganic nutrients, and metal transformations, applied to gaseous, aqueous and solid waste (Gavrilescu, 2004a).Intensification of agriculture and manufacturing industries has resulted in increased release of a wide range of xenobiotic compounds to the environment. Excess loading of hazardous waste has led to scarcity of clean water and disturbances of soil thus limit the crop production (Kamaludeen *et al.,* 2003). Bioremediation uses biological agents, mainly microorganisms, yeast, fungi or bacteria to clean up contaminated soil and water (Strong and Burgers, 2008). The match industry waste are highly polluted in close agreement with the by (Khalil, 2000) who studied textile effluent using *Pseudomonas aeroginosa* and *Bacillus subtilis.* The removal efficiency and the physico chemical parameters suggested the adoption of mono / single culture of fungus for bioremediation of match industry waste soil and this can also enhance ability to maintain the capacity of the isolates for bioremediation for extended periods of time and also make the application reusuable. The purpose of the present work was to investigate the ability of fungal isolates to accumulate the heavy metals and to use as bioremediating agent *in situ.*

The objection is the selection of the best microbe to be used in association with waste materials to reduce the heavy metals, with the advantage of best agent for bioremediation.

MATERIAL AND METHODS

Collection of match industry Waste soil

Match industry waste soil was collected from Sivakasi, Virudhunagar (Dt) and stored in gunny bags.

Measurement of Physico-chemical Parameters

The physico-chemical parameters of match industry waste soil such as pH, electrical conductivity, total dissolved solids, sodium, potassium, phosphate, chlorate and nitrogen were analysed using garden soil as control. Electrical conductivity (EC) was measured (Findlay and Kitchner, 1955); pH of the soil sample was measured with Elico (Popiel, 1972); sodium present in the sample was determined by Natusch and Hope, 1983; potassium by Natusch and Hope, 1983;

Isolation of fungi

Five fungi were isolated from the sample of match industry waste soil by serial dilution using Rose Bengal agar medium and pour plate technique. The pure cultures were made on PDA plates and identified by their morphological and colony characteristics (Onions *et al*., 1981) and the slants were sub cultured once a month.

Screening and selection of fungal isolates from match industry waste soil

Match industry waste (100g) was boiled in one litre of hot water (90°C) for 15 min. The supernatant of match industry waste was used to prepare one litre of match industry waste agar (MWA) medium by adding 15g of agar and sterilized at 121°C for 15 min and dispensed into Petri plates (20ml/plate). Fungal cultures were inoculated at the centre with a mycelial agar block (4mm) taken from the margin of 7 days - old fungal colony growing on PDA medium. The plates were incubated at 30 $\pm$ 2°C in the dark for 10 days. The radial growth of fungus was measured on potato dextrose agar medium (control) and match industry waste agar medium on the 10^{th} day after inoculation. The bleaching / removal of the colour at the reverse of the individual plates were observed on the 10^{th} day after inoculation.

Bioremediation of match industry waste in broth culture

Bioremediation of match industry waste was studied in liquid state in Erlenmeyer flasks (250ml) using the isolated fungus. Erlenmeyer flasks (250 ml) containing 100ml of potato dextrose broth (PDB) and match industry waste broth. The flasks were plugged with cotton and autoclaved at 121°C for 15 min. Single mycelial agar block (4 mm) from seven days-old cultures of the M_1, M_2, M_3, M_4 and M_5 isolated fungus was used as inoculum and were incubated at 30 ± 2^oC on a rotary shaker (120rpm) for 10 days.

Visual observations were made on the colonization / beaded formation of the respective fungus and match industry waste colour removal by the respective fungal treatments. All the experiments were carried out in triplicates and were replicated twice.

Based on their growth rate and colour removal of match industry waste soil both in Petri plates and flasks studies, the fungus namely *Aspergillus terreus* (M1) and *Aspergillus flavus* (M_2) were selected for further experimental studies.

RESULTS AND DISCUSSION

Soil microorganisms are ubiquitous in soils to which hyper accumulators are native, even in those soils containing high concentrations of metals (Schlegel *et al*., 1991; Ghaderian, *et al.,* 2000). Soil microorganisms can produce iron chelators and siderophores that ensure iron availability, reduce soil pH and / or solubilize metals phosphates (Abou-Shanab *et al*., 2003.)

Match industry waste soil is acidic; pH of the sample is 3.2. The low pH of the match industry waste soil is the consequence of the addition of chlorate powder. This is confirmative with the earlier reports of Sharma Sandhya *et al*., (2005).

In the present study, the match industry waste soil is brown in colour with chlorate odour. The dark brown colour of the match industry waste soil is due to the presence of raw material used. The Electrical Conductivity (EC) of the match industry waste soil is very high (Table 1). The increased electrical conductivity indicates the liberal use of inorganic chemicals in the manufacturing process. The amounts of sodium and chlorate ions are higher in the match industry waste soil. Match industry waste soil is found to contain low concentrations of major plant nutrients like nitrate and potassium but higher level of phosphate. This might be due to the use of chemicals in match industry.

Fungal isolates exposed to high levels of toxic elements in their environment have adapted to the stress by developing various resistance mechanism, these mechanisms could be utilized for detoxification and removal of toxic elements from polluted environment. The ability of fungal isolates to grow in the toxic elements would be helpful in the match industry waste soil treatment, where fungi are directly involved in the biological processes. The fungal isolates were identified as *Rhizopus stolonifer, Aspergillus niger, Aspergillus terreus, Penicillium funiculosum and Trichoderma harizianum* on the basis of their morphological and colony characteristics.

Growth rate of the fungal isolates in the presence of toxic elements (Sodium, Potassium, Zn, Sulphur) were consistently slower than that of the control both in PD agar medium and broth culture. In this study, fungal isolate M_1 and M_2 were highly resistant to toxic elements present in match industry waste soil were followed by M_3, M_4, M_5 in decreasing order respectively. According to the present study the isolated fungus may be used to remediate heavy metal contaminated match industry waste soil.

Growth rate of fungal isolates in the match industry waste soil was slower than that of the control. In this study M_3 (*Aspergillus flavus*) were highly resistant to toxic elements present in match industry waste and was followed by M_1, M_2, M_4, M_5 in decreasing order.

Biologically decolorization can be achieved by the use of naturally occurring microorganisms such as bacteria and fungi. In recent years, attention has been directed towards fungal decolorization systems (Moreira *et al*., 2000.)

Table 1. Physico-chemical parameters of match industry waste soil before and after treatment with fungal isolates.

S.No.	Parameter	Untreated match industry waste soil	Treated match industry waste soil	
			M1	M2
1	pH	3.2	5.6	6
2	TDS	3.8	2.5	2.3
3	Nitrate	63	30	27
4	Phosphorus	12.5	67	63
5	Potash	47.5	27.5	27
6	Sodium	2.8	2.3	2.1
7	Chlorate	1700	1100	980

Table 2. Identification of fungi isolated from match industry waste soil.

S.No.	Morphological characters	Colony characters	Identification
1	Colony growth : Fast Reverse of medium: Colourless.	Sporangiophores : Erect Spores: black.	*Rhizopus stolonifer*
2	Colony growth : Slow Reverse of medium: Yellow.	Conidiophores : Smooth, hyaline Conidia : Globose; smooth, brown.	*Aspergillus terreus*
3	Colony growth : Fast Reverse of medium: Colourless.	Conidiophores : Smooth; colorless Conidi: Rough; globose; green.	*Aspergillus flavus*
4	Colony growth : Slow,spreading Reverse of medium: Colourless.	Conidiophores : Short; arise from funicles Conidia : Green; elliptical;	*Penicillium funiculosum*
5	Colony growth : Slow,spreading Reverse of medium: Colourless.	Conidiophores : Short; arise from funicles Conidia : Light Blue	*Trichoderma harizianum*

Out of the 5 fungi isolated only 2 fungal species such as *Aspergillus terreus, A. flavus* showed the most prominent growth on medium with 10 %(w /w) matches industry waste soil. The growth rate of each fungal species was determined by measuring the radial growth for a period of 10 days after incubation at 30 $\pm 2^0$C.

The growth rate of these fungi which were isolated from the match industry waste soil was in decreasing order of M_1, M_2, M_3, M_4, and M_5 respectively (Table 3). The growth of *Aspergillus terreus* and *Aspergillus flavus* were not significantly affected in the 10% (w /w) match industry waste; but *Trichoderma sp* showed sensitivity towards 30 % (w /w) match industry waste soil. The same was supported by Razarmah *et al.,* (2011). They focused on a screening method for selecting the potential fungi which has the ability to be used in bioremediation of leachate (solid waste).

Through intensive study of lignolytic fungi, it has been determined that these organisms produce extracellular enzymes with very low substrate specificity. This makes them suitable for degradation of many different components, notably organo pollutants with structural similarities to lignin (PAH, PCBS, TNT, DDT) (Sarah Hamman, 2004). Three main genera of white rot fungi have shown potential for bioremediation are *Phanerochaete, Trametes, Pleurotus*.

Table 3. Growth (mm) of fungal isolates in Match Industry Waste Extract Agar Plates.

Fungal isolates	Radial (R) growth (mm)	Zone (Z) formation (mm)	Growth Ratio (mm) = Z/R
Aspergillus terreus	45	44	1.0
Aspergillus flavus	43	45	1.04
Penicillium funiculosum	32	36	1.12
Rhizopus stolonifer	37	42	1.13
Trichoderma harizianum	25	27	1.08

It is supported by Pointing 2001. He stated that white rot fungi have been shown to degrade a wide variety of environmental pollutants including penta chloro phenol.

Ashok kumar *et al*. (2010) revealed that microbes play a vital role in the remediation of heavy metal and other pollutant. In their study, the four samples out of sixteen were screened for biosorption study and the four microbial strains were *Pseudomonas sp.*

Staphylococcus sp., Bacillus sp. and *Aspergillus niger*. After treatment *Pseudomonas sp. Bacillus sp.* reduced Cu 4.165 mgl^{-1} 3.332 mgl^{-1} (68% and 56%) and Ni 5.015 mgl^{-1}(65% and 48%) respectively.

Aspergillus niger reduced Cd 0.267 mgl^{-1} (50%) and Zn 5.988 mgl^{-1}(58%) where as Staphylococcus sp. reduced Cr 4.108 mgl^{-1} (45%) Cu 2.615 $mgl^{-1}$and Pb 0.813 mgl^{-1} (93%). They showed that *Pseudomonas sp.* reduced heavy metals more than other microbes but *Staphylococcus* uptake the lead in very significant amount, it was measured that 93% of lead was reduced by *Staphylococcus* consequently, the *Pseudomonas sp*. which was isolated from soil was more potent bioremediation agent than other three microbes.

They concluded that microbes can tolerate against the heavy metals and there are armed with various resistance and catabolic potentials. This catabolic potential of microbes is enormous and is advandageous to mankind for the cleaner and healthier environment through bioremediation (Ashok Kumar *et al.,* 2010.)

Mycoremediation is an economically and environmentally sound alternative to extracting, transporting and storing toxic waste. One of the primary roles of fungi in the ecosystem is the decomposition, which is performed by the mycelium. The ligninolytic enzymes of white rot fungi are unspecific and thus, these fungi are considered to be potential microorganisms for bioremediation of polluted soils (Orth *et al*., 1994; Paszczynski and Crawford 1995; Hatakka 2001; Pointing 2001.)

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Corresponding author*:* Dr. K. Parani, Department of Botany with Specialization in Plant Biotechnology, The Standard Fireworks Rajaratnam College for Women (Autonomous), Sivakasi, Tamilnadu, India. Email: *parani_k@yahoo.co.in*