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BIODEGRADATION OF PHENOLIC COMPOUNDS USING FREE AND IMMOBILIZED FUNGI

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ABSTRACT

Biodegradation of phenolic compounds is one of the useful and highly effective methods to protect the global environment as phenol is the major effluents of many chemical industries, causing lethal effect to the human system. The present study has been carried out to check the decrease in COD levels of industrial effluent over a period of bioremediation and degradation of phenol content by use of free and immobilized cells of fungus isolated from different sources. Total of 9 isolates were obtained from different sources and only 3 were capable to degrade phenol red dye which were further identified on the basis of morphological and microscopic observations as *Aspergillus* sp., *Phaenerochaete* sp. and *Penicillium* sp. Chemical oxygen demand reduction and phenol degrading ability of these isolates were checked in free and in immobilized form. Percent reduction in COD after 120 hours of incubation by *Aspergillus* sp., *Phaenerochaete* sp. and *Penicillium* sp. was 72.37%, 68.57% and 44.36% respectively. Decrease in COD levels over a period of bioremediation indicated the degradation of phenol. Immobilized spores of *Aspergillus* sp., *Phaenerochaete* sp. and *Penicillium* sp. biodegraded 89.08%, 73.3% and 68.26% phenolic compounds whereas free spores biodegrade 68.91%, 64.3% and 61.28% of phenol respectively after 120 hours of incubation. The present study proved immobilized fungal isolates utilized phenol more rapidly as a sole carbon and energy source as compared to suspended forms and can be used for the removal of industrial contaminants.

Keywords: Fungi, COD, Phenolic compounds, Industrial waste, Biodegradation

INTRODUCTION

One of the major environmental problems faced by the world today is the contamination of soil, water and air by toxic chemicals. According to one survey eighty billion pounds of hazardous organopollutants are produced annually in the United States and only 10% of these are disposed of safely **[1]**. According to Environmental Protection Agency (EPA) list **[2]** (code U188), phenol is a major pollutant and toxic to all organisms and its lethal dose for human is reported as 5-10 mg/kg body weight. Phenol pollution is associated with pulp mills, coal mines, refineries, wood preservation plants and various chemical industries as well as their waste waters **[3]**. Natural sources of phenol include forest fire, natural run off from urban area where asphalt is used as the binding material and natural decay of ligno cellulosic material. The presence of phenol in water imparts carbolic odor to receiving water bodies and can cause toxic effects on aquatic flora and fauna **[4]**. The realization of high cost of physiochemical methods and ability of microorganism on phenol degration have opened door of bioremediation methods. Bioremediation is defined as the application of biological process for the treatment of pollution and is done by using naturally occurring **[5]** or by using genetically modified organisms **[6]** for treatment of soil, ground or surface water for general protection of human health and the environment. This has to be primarily safe and comparatively less expensive than conventional treatments. Most research within the field of bioremediation has focused on bacteria, but fungal bioremediation (mycoremediation) was attracting interest just within the past two decades as fungi are unique organisms that have capacity to degrade and mineralize lignin as well as organic, highly toxic and recalcitrant compounds **[7, 8]**. The extracellular lignolytic enzymes enable fungi to tolerate a relatively high conc. of toxic substances. Lignin has its structure similar to phenolic compounds and some fungi are lignin degrader. Similarity in the structure enables them to degrade phenolic compounds present in industrial effluent. The present study was aimed to check the biodegradation of phenolic compounds present in industrial effluent by application of fungi in relation to their cultivation mode, i.e. under the form of free mycelium and mycelium immobilized in alginate beads.

MATERIALS AND METHODS

Isolation and Identification of Fungi

Isolation from Soil and Sewage Sample

The serial dilution agar plating method is one of the commonly used procedures for the isolation of fungi **[9]**. Minimal salt media was used and isolates were maintained on potato dextrose agar (PDA).

Isolation from Rotten Fruits

It was done by using direct plating technique. Rotten fruits were collected and sterilized by washing with 70% ethanol, rinsed with sterile distilled water. Fruits were cut into small size using sterilized blade. PDA was poured into petriplates and allowed to solidify. To the semi-solid media the particle was plated using sterile forceps at the rate of 6-20 particles per plate. Inoculated petriplates were incubated upright at 25^0C for 3-5 days **[10]**.

Isolation from Fruiting Bodies Collected from Woody Substrate

Fruiting bodies of mushroom were collected from woody substrate and washed with distilled water. Exterior surface of fruiting body was sterilized by wiping with alcohol. The fruiting bodies were torn to expose the interior tissue and the tissues were quickly cutted into small pieces by using sterile scalpel and inoculated on PDA plates. The inoculated petriplates were incubated at 25°C for 3-4 days and monitored every day until mycelium developed **[11]**.

Screening for Lignin Degrading Ability by Phenol Red Analysis

Colonies obtained from different sources were checked for their phenol degrading ability. Use of dye enables visual detection of lignolytic activities and it was simple method of screening as no measurement is required **[12]**. Minimal Salt Media (MSM) was prepared and autoclaved for sterilization. Then MSM was cooled to room temperature, phenol red was mixed in media and media was poured onto petriplates. Hyphae of isolates were inoculated on plates and incubated. Plates were observed for growth and color change which indicates the ligninolytic activity of isolates.

Identification of Isolates

Morophological identification of isolates was done on the basis of colour, size, surface and rate of growth of colonies by following the standard methods **[13]**. Microscopic observations were done by staining with lacto phenol cotton blue using a compound microscope **[14]**.

Determination of COD (Chemical Oxygen Demand) of Effluent

For COD determination effluent sample was collected from village Ramnagar, district Patiala.

Immobilization of Isolates

Fungal spores were immobilized by using sodium alginate **[15]**. 2ml dispersed mycelium (OD-1.6) was mixed with 2%

sodium alginate and the mixture was dropped in sterile 0.1 M calcium chloride solution to form the beads containing the fungal spores. The formed beads were hardened at 37^0C for 15 min.

Biodegradation of Phenolic Compounds

Phenol degrading capacity of isolate was studied by using MSM **[16]**, containing carbon and nitrogen as a sole source for the growth of fungi. The free and immobilized spores of isolates were aseptically inoculated separately into 250ml conical flasks containing 100ml MSM. The flasks were incubated at 28^0C, for fungal growth **[17]**. Phenol was added to flasks, having immobilized and free fungal spores, for biodegradation, and OD of sample was observed at 362 nm at regular intervals. Phenol remaining in the medium was determined by using UV visible absorption spectrum.

RESULTS AND DISCUSSION

The main objective of this investigation was to use fungal isolates obtained from different sources and to degrade phenolic compounds present in effluent released from industries. About 9 different types of colonies were observed from different sources and 3 isolates showed lignin degrading activity by phenol red analysis. Based on morphological and microscopically observations these fungal isolates were identified as *Aspergillus* sp., *Phaenerochaete* sp. and *Penicillium* sp. **(Figure 1 and Table 1)**.

Percentage decrease in COD levels over a period of bioremediation was estimated which indicated the degradation of phenol by selected fungal isolates (**Figure 2**). After 120 hours of incubation, a maximum reduction in COD by *Aspergillus* sp., *Phaenerochaete* sp. and *Penicillium* sp. was 72.37%, 68.57% and 44.36% respectively. *Aspergillus* sp. was considered to be more effective in decreasing COD levels than other *Phaenerochaete* sp. and *Penicillium* sp. isolates. Hamdi *et al*., **[18]** reported 61% COD removal from OMW by *A. niger*. 79% reduction of COD content in OMW by *P. chrysosporium* was reported by Sayadi and Rad houane **[19]**. Chwei *et al*., **[20]** reported 35% of COD removal from OMW in batch culture by *A. niger*. Saritha *et al*. **[21]** observed that after 17 days of incubation, a maximum reduction in COD by *Phaenerochaete chrysosporium, Trametes hirsuta,* marine fungi and soil fungi was 73.54%, 79.6%, 66.4% and 47.6% respectively. Raja *et al*. **[22]** investigated that *Rhadotorula mucilaginosa* showed removal of COD from 95.68% to 56.71% whereas for *A. niger* the percentage varied from 98.02% to 69.51%. Yesilada *et al*. **[23]** used *Coriolus versicolor* and *Funalia trogii* to biodegrade

olive oil mill waste water (OOMW) and within 6 days, *C. versicolor* showed 63% COD reduction but *Funalia trogii* reduced COD of OMW upto 70 %. The phenol biodegrading capacity of both free and immobilized spores of different fungal isolates at different incubation time was presented in **Figure 3 and 4**.

The results obtained showed that immobilized spores were more efficient in removing phenol content than free spores. Immobilized spores of *Aspergillus* sp., *Phaenerochaete* sp. and *Penicillium* sp. biodegraded 89.08%, 73.3% and 68.26% of phenol whereas as free spores biodegrade 68.91%, 64.3% and 61.28% of phenol respectively after 120 hours of incubation. *Aspergillus* sp. is more efficient in biodegrading phenol in free and immobilized form as compared to *Phaenerochaete* sp. and *Penicillium* sp. Raja *et al.* **[22]** showed removal of polyphenolic compound from 83% to 45% by *R. mucilaginosa* and 94% to 58% by *A. niger* from olive mill wastewater. The result obtained was similar to those obtained by Kissi *et al.,* **[24]** who reported immobilized basidiomycetes filamentous fungi were able to remove more than 50% of phenol from olive mill waste. Pointing **[25]** showed that decrease in phenol content is due to release of multienzymes by the fungi. These enzymes have ability to degrade lignin and so they also have ability to degrade phenol. These results are in agreement with Gao *et al*., **[26]** according to whom, fungi contain various enzymes that degrade and mineralize organic/inorganic compounds.

Table 1: Morphological characteristics of hyphae of different isolates

Fungal Isolates	Colony Shape and Colour	Surface	Microscopic Characters	Presumptive Identification
Isolate -1	**Black brown colony, circular**	**smooth**	**Septate branched hyphae, Long conidia on conidiophores**	***Aspergillus* sp.**
Isolate -2	**White wooly, fuzzy colony**	**smooth**	**Septate hyphae, conidia on conidiophores**	***Phaenerochaete* sp.**
Isolate-3	**White mycelium, circular**	**margins deep, irregular**	**Multinucleate, septate hyphae, conidia are globose, branched conidiophores on the mycelia**	***Penicillium* sp.**

***Aspergillus* sp.**

***Phaenerochaete* sp.**

***Penicillium* sp.**

Figure 1: Fungal isolates from different sources

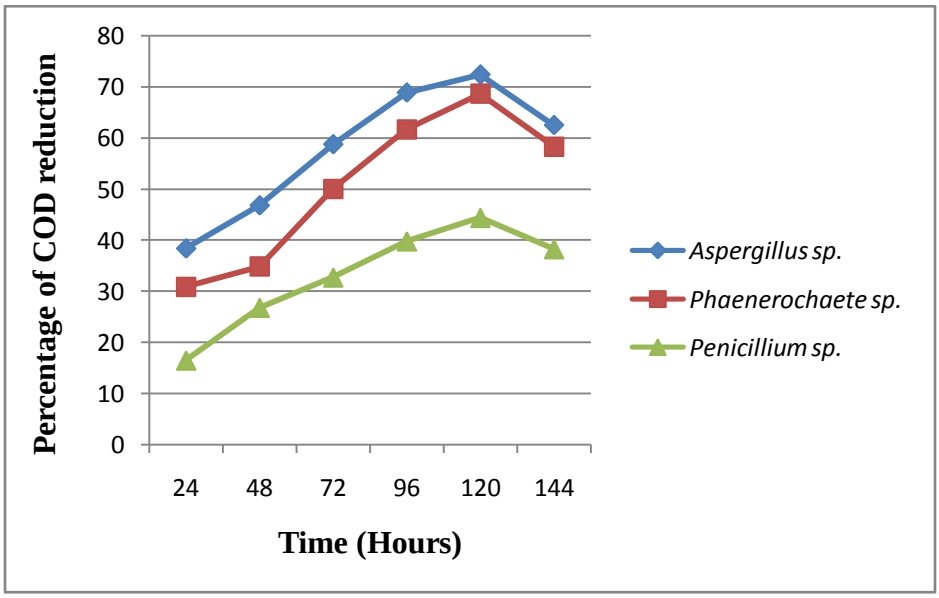

Figure 2: Decrease in COD level of effluent after treating with different isolates

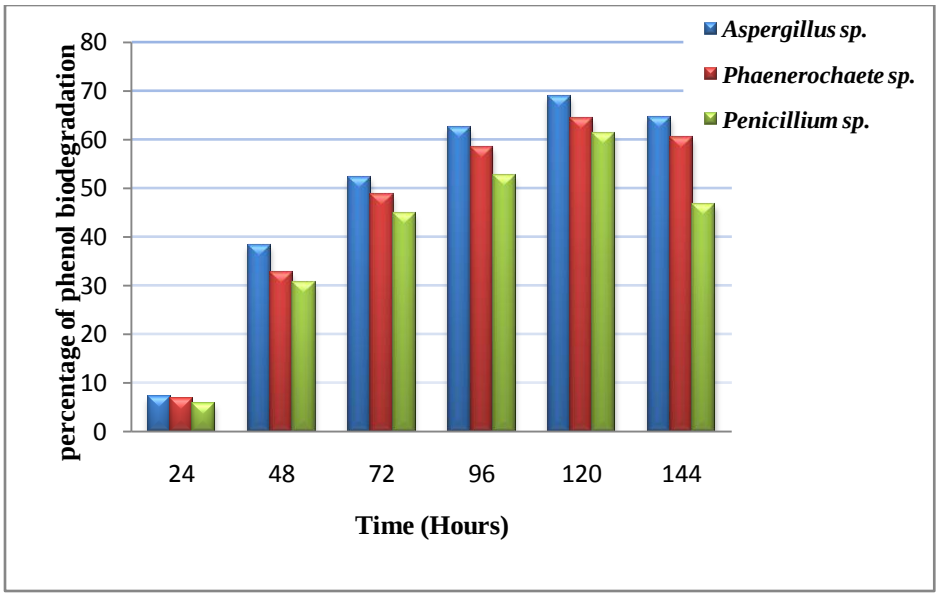

Figure 3: Biodegradation of phenolic compounds by free spores

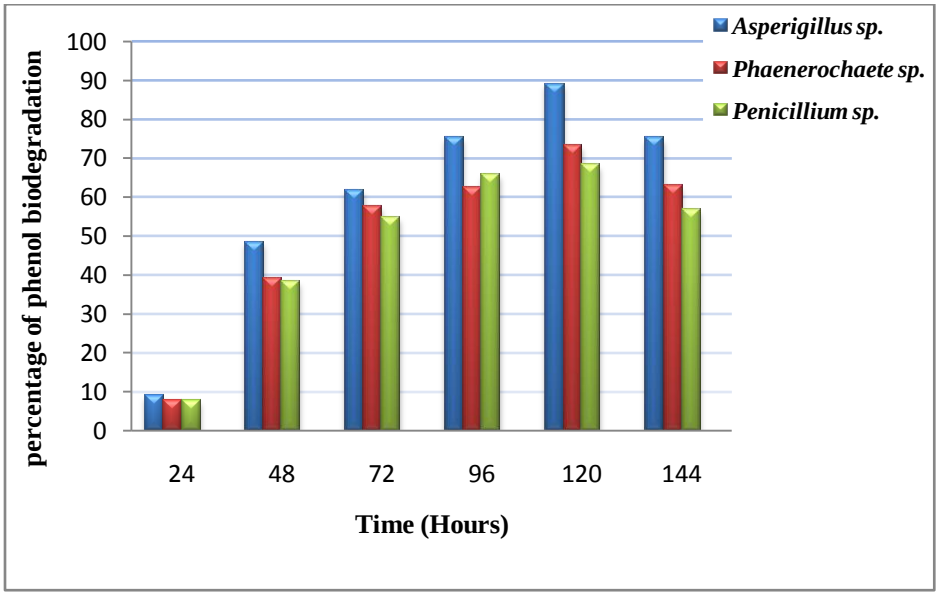

Figure 4: Biodegradation of phenolic compounds by immobilized spores

CONCLUSION

Phenolic compounds released by various industries in the environment, persist there for a long time and cause hazardous effect on the animals, plants, humans, etc. must be eliminated from the environment. Toxicity of industrial effluent is an important parameter that should be considered before its disposal into the environment. Present investigation revealed that fungus was able to reduce COD and phenolic compounds of industrial waste. Among the nine fungal isolates obtained from

different sources, only three isolates were able to degrade phenolic compounds which belonged to *Aspergillus* sp., *Phaenerochaete* sp. and *Penicillium* sp. Maximum reduction in COD occurred by *Aspergillus* sp. Immobilized spores can degrade the phenolic compounds more efficiently as compared to suspended form and *Aspergillus* sp. is more efficient in biodegrading phenol in free and immobilized form as compared to *Phaenerochaete* sp. and *Penicillium* sp. However, further studies are needed to evaluate mass production of the fungus for optimal bioremediation of industrial wastes. Although slow, on the whole microbial bioremediation was found to cover wide range of recalcitrant degradation and is known to be a better choice because of its nature of degradation.

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