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Biodegradation of Xenobiotic (phenol) by using *Aspergillus niger* isolated from waste fruits

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ABSTRACT

The present investigation enphasized the biodegradation of xenobiotics like phenol by using *Aspergillus niger* isolated from waste fruits. Phenolic compounds are hazardouse pollutants that ae toxic to the natural ecosystem at very low concentration. Biodegeradation is the process by which organic substances are broken down into smaller compounds by the catalytic activity of living microbial organisms. The percentage of phenol degeradation in 40µl flask after 3rd day was 10%, after 7th was 51.60%, after 8th was 73.33%. The maximum phenol degeradation was observed at 8th day. The percentage of phenol degeredation in 60µl flask after 3rd day was 21.87%, after 7th day was 69.53%, after 8th day was 77.34%. When compared the result of 40µl and 60µl phenol concentration, the maximum phenol degeradation was observed in flask containing 60µl phenol concentration. No phenol degeradation was observed in 100µl flask. Biodegeradation is completely safe and ecofriendly. Biodegeradation not cause any type of pollution and also a cheap method so economically also benificial.

Key words : Biodegradation, Xenobiotcs, Phenol, *Aspergillus niger*, waste fruits

INTRODUCTION

Due to rapid industrialization and economic development, many pharmaceutical and chemical industries are releasing their effluents in to natural ecosystem and they are inhibiting the sunlight penetration and reducing the photosynthetic activity of aquatic system. Many of these aromatic compounds are toxic to the living system and their presence in the aquatic and terrestrial habitats often have serious ecological consequences. Among all these aromatic compounds phenol is the most toxic compound and it can persist in the environment for long time due to its long range transporation, bioaccumulation in human and animal tissue and biomagnification in food chain. Biodegradation is emerging as most

ideal technology for removing phenolic pollutants from the environment by the action of microbes restoring contaminated sites and preventing further pollution (Atlas *et al.* 1996).

Interest in the microbial biodegradation of pollutant has intensified in recent years as mankind to find sustainable ways to clean contaminated environment. Large amount of aromatic compound are synthesized by our ecosystem. Waste from industries creates additional problem. Most serious problem arises due to hazardous chemicals, metals, metalloid without proper treatment that reaches water body causing bioaccumulation and killing of life forms. In addition it reaches the water table, and is distributed a long way causing community diseases and poisoning. (Sharma *et al.* 2012) Water pollution caused due to the release of waste product and contaminant leaching into ground water. One of the major problems is soil contamination. It occurs due to the release of chemicals by spills of underground leakage or also by hydrocarbons, chlorinated hydrocarbon (Tridedi 1994). These bioremediation and biotransformation method endeavour to harness the astonishing, naturally occurring, microbial, catabolic diversity to degrade, transform or accumulate huge range of compound including hydrocarbon, poly aromatic hydrocarbon. Biodegradation is the breakdown of organic contaminant that occur due to microbial activity. As such, these contaminant can be considered as the microbial food source or substance (Gönül *et al*. 1999).

Complete biodegradation or mineralization involves oxidation of the parent compound to form carbon dioxide and water, a process that provides both carbon and energy for growth and reproduction of cells. Each degradation step in the path way is catabolised by a specific enzyme mode by the degrading cell. Incomplete degradation in which a partial oxidation of the substrate occurs but the energy derived from oxidation is not used to support microbial growth (Raina *et al.* 2000).

Biodegradable matter is generally, organic material such as plants and animal matter and other substances origination from living organism or artificial material that are similar enough to plants and animals matter to be put to use by microorganism. Some microorganism have the astonishing, naturally occurring microbial catabolic diversity to degrade, transform or accumulate huge range compound including hydrocarbon, poly aromatic hydrocarbon etc. New methodological bank through in sequencing, genomic, proteomic, bioinformatics and imaging are producing vast amount of information (Varsha *et al.* 2011).

Biodegradation of aromatic compound :

Under natural condition, the release of carbon atoms from aromatic ring involvement depends in environmental and ecological condition. The aromatic compounds of both natural and manmade sources abound in the environment. The aromatic compound can be completely degrade under anaerobic conditions. If the aromatics is oxygenated clearly, higher redox potential are required for degradation of more complex substrate such as benzoate, leaving smaller organic acid or alcohol molecules that are degraded at lower redox potential (Raina *et al.* 2000).

Phenols are introduced in environment in waste water stream of several industrial operations, through its use as antimicrobial agent or as a by- product of other pharmaceutical industries, or even waste incineration and as degradation product of other chlorinated xenobiotics. Phenol is a aromatic hydrocarbon also known as carbolic acid. Phenol has molecular formula $C_6H_{12}OH$. The molecule consists of a phenyl group bonded to hydroxyl group. It is mildly acidic and requires careful handling due to its propensity to cause chemical burns (Wackett *et al.* 2001). Phenol pollution is associated with pulp mills, coal mines, refineries, wood preservation plants and various chemical industries as well as their waste water (Paula *et al.*1995). 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 cellulosic material. The presence of phenol in water imparts carbolic odour to receiving water bodies and can cause toxic effects on aquatic flora and fauna (Gandhi *et al.* 1995). Phenol are toxic to human beings and affect several biochemical functions (Nuhoglu *et al.* 2005).

Inspite of phenolic toxic properties, a number of microorganism can utilize phenol, under aerobic condition as sources of carbon and energy (Chen *et al.* 2006). Biodegradation technologies most often take advantage of the ability of various bacteria or fungi to clean the environment bioremediation are constantly expanding. Fungi are famous for their wide incidence and the outstanding capacity of degrading complex and inert natural products like lignin, chitin and cellulose. Fungi adopt more easily than bacteria and are capable to grow in extreme conditions, like nutrients deficiency, low pH, limited water supply, etc (Atagana, 2000) and not on the least, there comes the ability of fungi to survive in the presence of various xenobiotics that turn to be toxic to a number of microorganisms. Metabolism of aromatic compounds, phenol and its derivatives, has been extensively researched in prokaryotic microorganisms (Watanabe *et al.* 1998). Particularly huge information have been collected about bacterial species of *Pseudomonas* genus (Seker *et al.* 1997). Number of individual representatives of genera *Candida, Rodotorula* and *Trichosporon*, which are capable of metabolising aromatic compounds (Cerniglia *et al.* 1981). The specific enzymes responsible for biodegradation occupy an important place in these investigation. From *Penicillin, Aspergillus, Fusarium, Graphium* and *Phanerochaete* genera to disintegrate aromatic compounds. In 15 strains belonging to *Fusarium, Aspergillus, Penicillium* and *Graphium* genera the presence of phenol hydroxylase and catechol 1,2-dioxygenase activity in cells cultivated on phenol containing medium has been confirmed. These finding demonstrate that catechol oxidation follows the ortho-pathway of breaking the aromatic ring. Mostly used strain for biodegradation of phenol is *Aspergillus niger. Aspergillus niger* is a fungus and one of the most common species of the genus *Aspergillus*. It causes a disease called black mold on certain fruits vegetables such as grapes, onions, peanuts, andit's a common contamination food. it is ubiquitous in soil and is commonly reprted in indoor environments, where its black colonies can be confused with those of *Stachybotrys.*

MATERIALS AND METHODS

I] Collection of waste fruit from fruit market

The waste fruit (onion) was collected from cotton market, Nagpur.

II] Isolation and identification of *Aspergillus niger*

The waste fruit were collected. Potato dextrose Agar was prepared, sterilized & solidified. The black portion on the surface of onion was inoculated into potato dextrose Agar in a petri plate the whole process carried out aseptically. The plates were inoculated for 7 days at room temperature at 28^0C. **A**fter 7 days the plates were observed for mycellial growth.

Aspergillus niger was isolated from waste fruits and was maintained on potato dextrose agar (PDA) at 4^0C. For mass culturing, the liquid broth was used as a culture medium which was having composition (gl^{-1}): 20 Dextrose; 10 Peptone; 0.2 NaCl; 0.1 $CaCl_2.2H_2O$; 0.1 KCl; 0.5 $NaHCO_3$; 0.25$MgSO_4$ and 0.005 $FeSO_4.H_2O$. The liquid phase pH was adjusted to 4.5 by using the 0.1M HCl and 0.1N NaOH. The liquid broth was inoculated with a loop of culture grown on PDA medium and incubated on an orbital shaker at 125 rpm and 25^0C for 5 days in 500ml conical flasks. The biomass produced was collected by filteration and washed twice with extra pure double distilled water (Sharma *et al.* 2012).

III] Morphological characteristics:

In case of fungi to study the morphology lactophenol cotton blue staining were carried out.

Cotton blue staining (Lactophenol cotton blue) :

Place a drop of seventy percentage alcohol on a clean microscope slide. Materials from cultures of filamentous fungi should be removed using a stiff inoculating wire not the loop used for manipulations with bacteria or yeasts. Remove a small amount of the culture. For fungal cultures, it is often useful to take a little of the agar medium together with the fungus. In any case, the material should be disturbed as little as possible when being transferred to the slide. Immerse the fungal materials in the drop of 70% alcohol. This drives out the air trapped between the hyphae. Scrap out the material very gently with mounted needles. Fungal structures are readily visualised after staining with a lactophenol cotton blue dye preparation. Before the alcohol dries out add one or at most two drops of the stain. Lower the coverslip gently onto the slide, trying to avoid air bubbles. Your preparation is now ready for examination. Switch to a higher power 40X objective for more detailed examination of spores and other structure (Kerina *et al.* 2002).

IV] Preparation of inoculum:

The isolates were maintained on potato dextrose agar slants. Generally 72hrs old culture was used for the preparation of inoculum. The strain was propagated in a PDA agar slants and maintain at 35^0C for 5 days until sporulation takes place. The slants which shows the maximum growth of fungal organism was used in the phenol degradation study (Ch. Superiya *et al.* 2014)

V] Fungal spore suspension:

Spores suspension was prepared in sterilized distilled water. The spores from potato dextrose agar slant containing *Aspergillus niger* was inoculated in conical flask containing sterilized distilled water (Sharma *et al.* 2012).

VI] Biodegradation of Phenol :

The phenol degradation experiments were carried out by taking three conical flasks. This three conical flask contain *Aspergillus niger* spore suspension on equal amount (1000µl), but the phenol concentration varies. The flask contained 60µl, 80µl and 100µl concentration of phenol. Potato dextrose media was prepared and autoclaved than it added in each flask. The *Aspergillus* spore was inoculated into the three flasks in equal concentration. Addition of phenol in different concentration in each flask first flask contained 60µl phenol second flask contained 80µl phenol and the third flask contained 100µl phenol. Three control conical flask was also used which contained 60, 80, 100µl phenol without spores of *A. niger*. This all flasks were incubated at room temperature for 8 days. Samples were withdrawn in 3^{rd}, 7^{th}, 8^{th} day for phenol determination. The sample was withdrawn aseptically and centrifuge at 12,000rpm. After centrifugation the sample subjected to phenol determination studies (Ch. Superiya *et al.* 2014).

VII] Determination phenol degradation :

For phenol determination the Folin-Ciocalteau phenol method was used, involving the successive addition of 1 ml sodium carbonate (200mg/l) and 0.5 ml Folin Ciocalteau phenol reagent to 10 ml sample. After 60 minutes at 20^0C, the absorbance was measured at 625 nm against control flask used as a blank (Sharma *et al.* 2012).

The biodegeradation of phenol measured using colorimeter

Formula of percent decolorisation:

$$\% \text{ Decolorisation} = C_0 - C * 100 / C_0$$

Where,

C_0 is the initial concentration of phenol

C is the concentration of phenol after degradation

RESULTS & DISCUSSION

Phenol degradation was carried out using *Aspergillus niger*. *Aspergillus niger* was isolated from waste fruit (onion) collected from cotton market nagpur. Now with the help of inoculating needle the ssmall mass of waste fruit picked and inoculated in potato dextrose agar plate this plate is incubated for 7 days in room temperature. After 7 days the black colour colonies was observed in the petri plate. Cotton blue staining was carried out for morphological analysis, mycelium was observed under 100X microscopy.

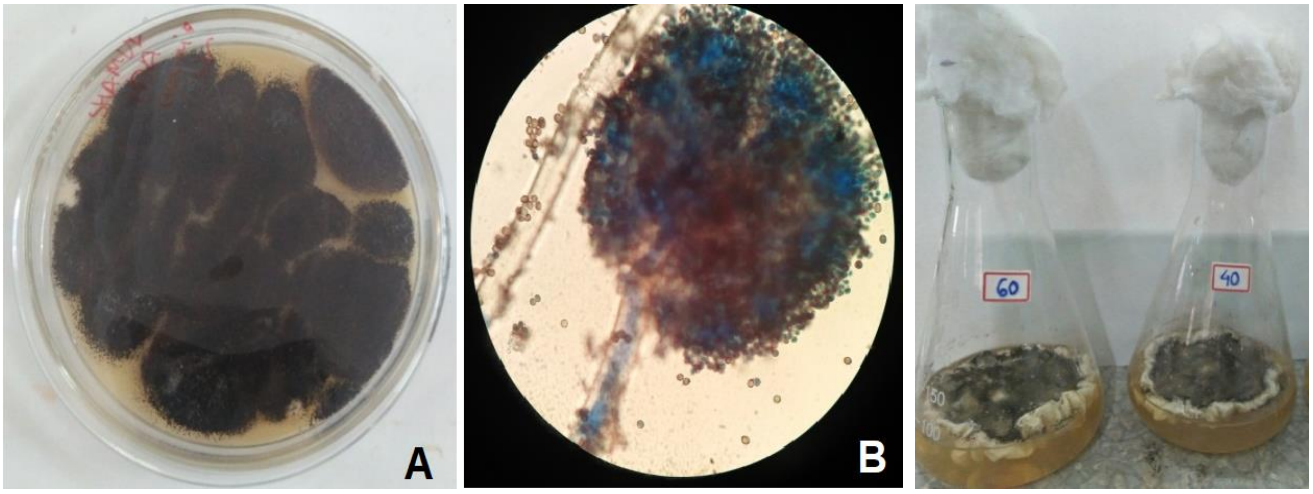


Fig. 1 : Morphological characteristics of *A. niger* by cotton blue staining

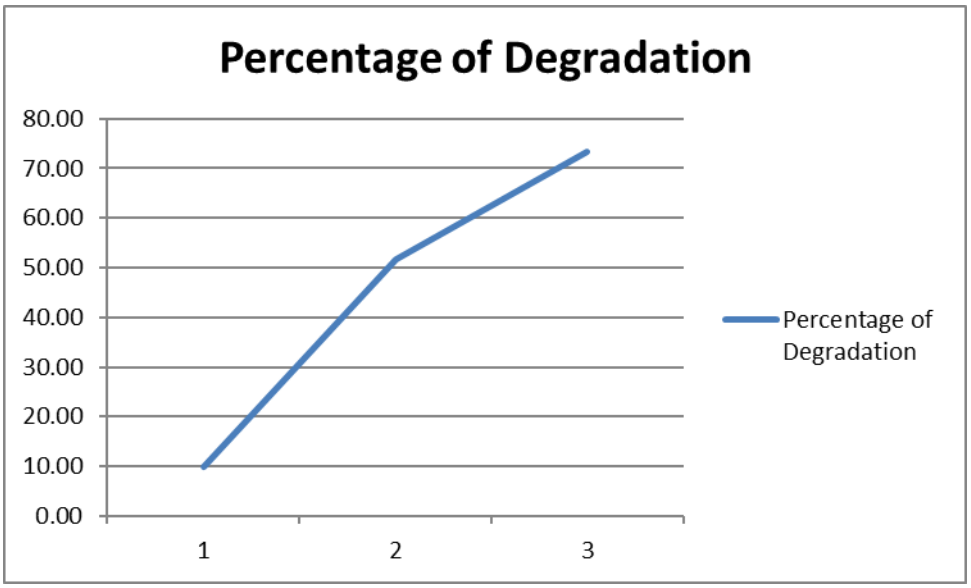
Fig. 2 : Sporulation of *A. niger* after 8th days of incubation

Spore suspension of *A. niger* was prepared in double distilled water in aspetic condition. In Potato dextrose broth was added in 250ml conical flasks than different concentration of phenol was added in each flask such as 40µl, 60µl and 100µl aseptically. Spore suspension of *Aspergillus niger* was inoculated. Three flask are controlled contain potato dexrose broth and phenol in different concentration. This flask incubated for 3rd, 7th, 8th days at room temperature. White colour mycelium was observed in 3rd day in two flask (40µl, 60µl). Complete sporulation occur at 8th day in two conical flask 40µl and 60µl. Black colour mycelium was observed.

Determination of Phenol Degradation :

The phenol determination was carried out using spectroscopy (660nm). The percentage of phenol degeradation in 40µl flask after 3rd day was 10%, after 7th was 51.60%, after 8th was 73.33%. The maximum phenol degeradation was observed at 8th day. The percentage of phenol degeredation in 60µl flask after 3rd day was 21.87%, after 7th day was 69.53%, after 8th day was 77.34%. When compared the result of 40µl and 60µl phenol concentration, the maximum phenol degeradation was observed in flask containing 60µl phenol concentration. No phenol degeradation was observed in 100µl flask. [Table No. 1]

Sr. No.	Concentration of Phenol in µl/100ml	Incubation Days	O.D at 660nm	Percentage of Degradation
1	40µl/100ml	3	1.01	10%
		7	0.58	51.6%
		8	0.32	73.33%
2	60µl/100ml	3	1.00	21.87%
		7	0.39	69.53%
		8	0.29	77.34%



Ywetta Maletero, *et al.,* (2016) worked on biodegeradation of phenol by using two different microorganisms *Candida Tropicalis* and *Phanerochaete chysporium*. The maximum average value of phenol biodegeradation rate in aqueous solution obtained at the initial concentration of phenol of about 300mg/l was 12.2 mg/l/h at cell density of 0.025g/l. Supriya and Deva (2014) worked on biodegeradation of phenol using aspergillus niger they used different concentration of phenol such as 100mg/l, 200mg/l, 300mg/l, 400mg/l and 500mg/l each flask having 5mg mycelium mass of *Aspergillus niger*. After incubation for 120days the maximum phenolic degerdation was observed at 300mg/l this is due to the fact that phenol degerading enzyme activity is optimum at this concentration.

Bui et al. (2012) reported biodegradation of Phenol by Native Bacteria Isolated From Dioxin Contaminated Soils. The results showed that these bacteria were highly effective for the removal of phenol. After 120 hours of culture, strain D1.4 degraded 54.84% and 44.19% phenol from the initial concentrations of 100 mg/L and 1000 mg/L, respectively; strain D1.6 degraded 66.45% of phenol from the initial concentration of 1500 mg/L. Sharma *et al*., (2012) worked on biodegradation of phenol by a fungal isolate *Aspergillus niger* was studied a batch flask system with synthetic and industrial effluent. *Aspergillus niger,* was efficiently immobilized on sodium alginate beads. The immobilized cells were used in the batch culture flasks for paper and pulp industry as well as synthetic effluent phenol removal. All the flasks were operated at temperature 25^0C at 125 rpm for five days in continuous mode. The immobilised cells showed over all better performance as compared to free cells. The average overall pH, Temp., conductivity, B.O.D, C.O.D., Chloride and phenol were upto 7.5, 34.75^0C, 39.6 µmol/cm, 139.5 mg/l, 430.5 mg/l, 1490 mg/l, 900 mg/l, 590mg /l, 281.25 mg/l, 268 mg/l respectively. While culture with immobilized cell reached 110 mg/l whereas in free cell it is 119 mg/l in industry effluent culture with immobilized cell reach 28 mg/l whereas in free cell it is 150 mg/l with the same conditions. Reduction in phenol level proved the biodegradation. Tebbouche, *et al.,* (2015) worked on evaluation of phenol biodegradation by *Aspergillus niger*. This study indicates the excellent ability of *Aspergillus niger* to degrade different concentration of phenol in batch tests. The maximum rate of phenol elimination was 1.32 mg/h and this optimum was achieved at 200mg/l initial phenol concentration, pH 8.5 and 35^0C. Biodegeradation is completely safe and ecofriendly. The organism used in this study is also ecofriendly means not produced any toxic compound during biodegeradation. Biodegeradation not cause any type of pollution and also a cheap method so economically also benificial.

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