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DECOLORIZATION OF SYNTHETIC DYES USING SPENT MUSHROOM SUBSTRATE

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#### Abstract:

The present study based on the de-colorization of synthetic dye by fungal strain isolated from Spent Mushroom Substrate (SMS). The Spent Mushroom Substrate is by-product of mushroom industry bearing many fungal strains having capacity to decolorize synthetic dye. Out of the seven fungal strains isolated from SMS, five strains showed de-colorization and five synthetic dyes used in which three were de-colorized by isolated fungal strain. This study shows that dye de-colorization using Spent Mushroom Substrate is a non-conventional and less costly method than chemical method.

### Introduction:

Removal of dye colour during water treatment process are very tedious and time consuming. Although various chemical and biochemical method available for decolourization of synthetic dye present in water but it is very costly and long lasting process. So we have to find some alternative effective method. Spent Mushroom Substrate has capability to de-colorize synthetic dye. Spent Mushroom Substrate is by product of Mushroom Industry.

Today mushrooms are becoming more and more popular amongst people as a continental or Chinese delicacy. Mushroom industries generate a virtually exhaustible supply of co-product called Spent Mushroom Substrate. This is an unutilized substrate and the mushroom mycelia left after harvesting of mushroom. As the mushroom industries are steadily growing, the volume of SMS generated annually is increasing. In recent years, mushroom industry has faced challenge in storing & disposing the SMS. The use of solid residue from mushroom is adsorbing and decolorizing different dyes. The application of this SMS for wastewater treatment will be able to take advantage of both Mushroom Industry and water treatment industry for dye removal processes.

#### Materials and Method: Materials

Synthetic dyes used were Methyl orange, Methylene blue, Leishman's stain, Crystal violet and Saffranin. Culture media used were Potato Dextrose Broth (PDB) & Potato Dextrose Agar (PDA) to grow the fungal isolates. Spent Mushroom Substrate (SMS) were collected from Mushroom cultivation factory

### <u>Method</u>

### Enrichment of fungi from SMS:

Isolation of dye decolorizing fungi from SMS was carried out by enriching the SMS suspension. 10g of spent mushroom substrate suspended in 90ml of Potato Dextrose Broth (PDB). The mixture was incubated at 37° C for four days

# Isolation of Dye de-colorizing Fungi from SMS inoculums:

Potato Dextrose Broth (PDB) containing synthetic dyes is inoculated with 1ml SMS inoculum. The Mixture is incubated at 37° C for four days. The dyes which show decolourization after incubation period were used for further procedure. The dyes which shows decolourization after incubation period was added in Potato Dextrose Agar (PDA) and the medium was autoclaved. The Potato Dextrose Agar (PDA) plates after autoclaving was inoculated with SMS inoculum by pour plate method. The plates are incubated at 37° C for four days. After incubation period, the colonies showing maximum de-colorizing zones were selected and collected on separate Potato Dextrose Agar (PDA) slants. The collected fungal isolates from SMS were developed on Potato Dextrose Agar (PDA) after four days of incubation & used for further study.

# De-colorization experiments using fungal isolates from SMS:

The collected fungal isolates from SMS were put on separate de-coloriztion experiments. Potato Dextrose Broth (PDB) containing synthetic dyes were inoculated with fungal isolates from SMS. The tubes were incubated at 37° C for four days. The results were observed after incubation period.

**De-colorization experiments on increasing concentration of synthetic dyes:** The collected fungal isolates from SMS were put on separate de-colorization experiments on increasing concentration of synthetic dyes. Potato Dextrose Broth (PDB) containing synthetic dyes were inoculated with fungal isolates from SMS on increasing concentration of synthetic dyes. The tubes were incubated at 37° C for four days. The results were observed after incubation period. The Optical Diffraction of de-colorized broth was calculated using colorimeter. Graphs were plotted. Results of isolates were compared by charts.

## **Results and Discussion:**

The spent mushroom substrate inoculum was prepared after 4 days of incubation which then inoculated with Potato Dextrose Broth containing different synthetic dyes. Out of five synthetic dyes used in this study, Methyl orange, Methylene Blue and Leishman's stain was found to be decolorized after incubation period, Saffranin and Crystal violet was not found to be decolorized. When the spent mushroom substrate inoculum was screened on Potato Dextrose Agar medium containing synthetic dyes, the results observed after incubation period were as Methyl orange, Methylene Blue and Leishman's stain was found to be decolorized and Saffranin and Crystal violet was not found to be decolorized. From those plates seven different isolates which shows zone of de-colorization were collected on separate slants and studied separately for de-colorization. The seven isolates obtained from spent mushroom substrate are as follows:

- 1. Isolate A Colorless from Methylene Blue
- 2. Isolate B Green from Leishman's Stain
- 3. Isolate C Sticky from Methylene Blue
- 4. Isolate D Small Pink from Methylene Blue
- 5. Isolate E Colorless from Leishman's Stain
- 6. Isolate F Pin point from Methyl Orange
- 7. Isolate G Cream color sticky from Methyl Orange

When isolate A was studied for separate decolorization it was observed that it did not decolorizes dyes. When Isolate B studied for separate de-colorization it was observed that it decolorizes Methyl orange, Methylene Blue and Leishman's stain & did not decolorized Saffranin and Crystal violet. When the three dyes which showed de-colorization were further studied at increasing concentration, the result observed that at low concentration of dye, the de-colorization rate was high & shows lowest optical density & at high concentration of dye, the de-colorization rate was low shows highest optical density. When Isolate C studied for separate de-colorization it was observed that it decolorizes Methyl orange, Methylene Blue and Leishman's stain & did not decolorized Saffranin and Crystal violet. When the three dyes which showed decolorization were further studied at increasing concentration, the result observed that at low concentration of dye, the de-colorization rate

was high & shows lowest optical density & at high concentration of dye, the de-colorization rate was low& shows highest optical density. When Isolate D studied for separate decolorization it was observed that it did not decolorizes dyes. When Isolate E studied for separate de-colorization it was observed that it decolorizes Methyl orange, Methylene Blue and Leishman's stain & did not decolorized Saffranin and Crystal violet. When the three dyes which showed de-colorization were further studied at increasing concentration, the result observed that at low concentration of dye, the de-colorization rate was high & shows lowest optical density & at high concentration of dye, the de-colorization rate was low & shows highest optical density.

When Isolate F studied for separate decolorization it was observed that it decolorizes Methvl orange, Methylene Blue and Leishman's stain and did not decolorized Saffranin and Crystal violet. When the three dyes which showed de-colorization were further studied at increasing concentration, the result observed that at low concentration of dye, the de-colorization rate was high and shows lowest optical density and at high concentration of dye, the de-colorization rate was low and shows highest optical density.

When Isolate G studied for separate decolorization it was observed that it decolorizes Methyl orange, Methylene Blue and Leishman's stain and did not decolorized Saffranin and Crystal violet. When the three dyes which showed de-colorization were further studied at increasing concentration, the result observed that at low concentration of dye, the de-colorization rate was high and shows lowest optical density and at high concentration of dye, the de-colorization rate was low shows highest optical density.

The isolates which show decolorization of Methyl orange were compared at concentration 1.5/ml, it was observed that Isolate E shows highest rate of de-colorization & lowest optical density. Higher is the rate of de-colorization, lower is the optical density. Isolate F shows lowest rate of de-colorization & highest optical density. Lower is the rate of decolorization, higher is the optical density. Methyl orange were compared at concentration 2/ml, it was observed that Isolate E shows highest rate of de-colorization & lowest optical density. Isolate F shows lowest rate of decolorization & highest optical density.

Methylene Blue were compared at concentration 1.5/ml, it was observed that Isolate B shows highest rate of de-colorization & lowest optical density. Isolate E shows lowest rate of de-colorization & highest optical density. Methylene Blue were compared at concentration 2/ml, it was observed that Isolate B shows highest rate of de-colorization & lowest optical density. Isolate E shows lowest rate of de-colorization & highest optical density.

Leishman's stain were compared at concentration 1.5/ml, it was observed that Isolate E shows highest rate of de-colorization & lowest optical density. Isolate F shows lowest rate of de-colorization & highest optical density. Leishman's stain were compared at concentration 2/ml, it was observed that Isolate E shows highest rate of de-colorization & lowest optical density. Isolate C shows lowest rate of de-colorization & highest optical density.



### **Conclusion:**

This study shows that out of the different isolates obtained from spent mushroom substrate, Isolate B, Isolate C, Isolate E, Isolate F & Isolate G shows de-colorization and Isolate A & Isolate D do not show de-colorization.

The rate of de-colorization of some isolates is higher & the rate of de-colorization of some isolates is lower. At low concentration of dyes, some isolates shows high rate of decolorization & at high concentration of dyes, some isolates shows low rate of decolorization. Further study is required before establishing any judgment. Adsorption of dyes to the microbial cell surface is the primary mechanism of decolourization. In our study, the adsorption of dyes by the fungal Mycelium was also observed, as it was confirmed by the change in the color of fungal mycelium in tested dyes.

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