

## OPTIMIZATION STUDIES ON POLLUTION ABATEMENT: BIODEGRADATION OF NITROSO DYE EFFLUENTS BY TWO FUNGI (*PHANEROCHAETE CHRYSOSPORIUM* & *TRAMETES HIRSUTA*) UNDER STATIC CONDITIONS

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### ABSTRACT

Biodegradation of Nitroso dye effluent by two fungi viz., *Phanerochaete chrysosporium* and *Trametes hirsuta* was carried out. These two fungal strains showed considerable decolorization, BOD and COD removal efficiently from dye effluent. Tropically grown edible mushroom *Trametes hirsuta* degraded nitroso dye without much input of energy. In vitro studies showed complete decolorization of the nitroso dye by these organisms within 25 days as revealed by spectrophotometric analysis.

**Keywords:** Nitroso Dye, *Phanerochaete chrysosporium*, *Trametes hirsuta*.

### INTRODUCTION

In a quest of development, man is hampering the ecological balance, particularly, in the later half of twentieth century. Today we do not have fresh air to breathe; pure water to drink, quiet atmosphere to live peacefully and the environment is in serious jeopardy. The developing countries, like India has established large number of industries even in the rural area to improve the economic status, but they fail to give importance to sound environmental management systems. This has been the root cause for the increasing pollution. The major pollution causing industries include Textile Dyeing, Tannery, Paper and Pulp, Food processing, Dairy, Electro-plating, Distilleries, Pharmaceuticals and Dye industries<sup>1</sup>. These industries consume about 4000 million Liters of water and generate about 3000 million Liters of effluents every day<sup>2</sup>.

To carryout present work, two textile-dyeing units were selected the detailed information regarding the type of dye, method of dyeing, quantity of dye used and the amount of waste produced during the day were collected.

### MATERIAL AND METHODS

#### Collection of Dye Effluents

Dye effluents were collected both at The Rainbow Cloth Dyeing and Printing Works, Lakshmi Sai Banaras saree polishing printing and dyeing works during dyeing period. The effluent is collected in white plastic can and the samples were brought to laboratory with necessary precautions and labeled suitably. Parameters like Temperature, pH and dissolved Oxygen (D.O) are measured onsite as they are liable to change with time. The Physico-chemical parameters of the pre and post biodegradation assay effluent were analyzed by adapting standard procedures from manual of American Public Health Association<sup>3</sup>. Pure culture were made

#### Biodegradation Studies

The pure cultures of *Phanerochaete chrysosporium*, and *Trametes hirsuta* were inoculated in 50 ml Potato Dextrose Agar and Rose Bengal medium in laminar air flow. After inoculation the culture bottles were incubated at 28 ±1°C. The growth of fungus appeared after 48 hours of inoculation. These were inoculated with oil-contaminated soils and CO<sub>2</sub> release was estimated.

The work was further extended by inoculating the fungal species in two sets of soil sample of which one is sterilized set of two conical flasks into which *Phanerochaete chrysosporium* and *Trametes hirsuta* were inoculated and into other set consisting of three conical flasks one was kept with unsterilized soil with its indigenous

species and the other two flasks with unsterilized soil were inoculated with *Phanerochaete chrysosporium*, and *Trametes hirsuta* respectively.

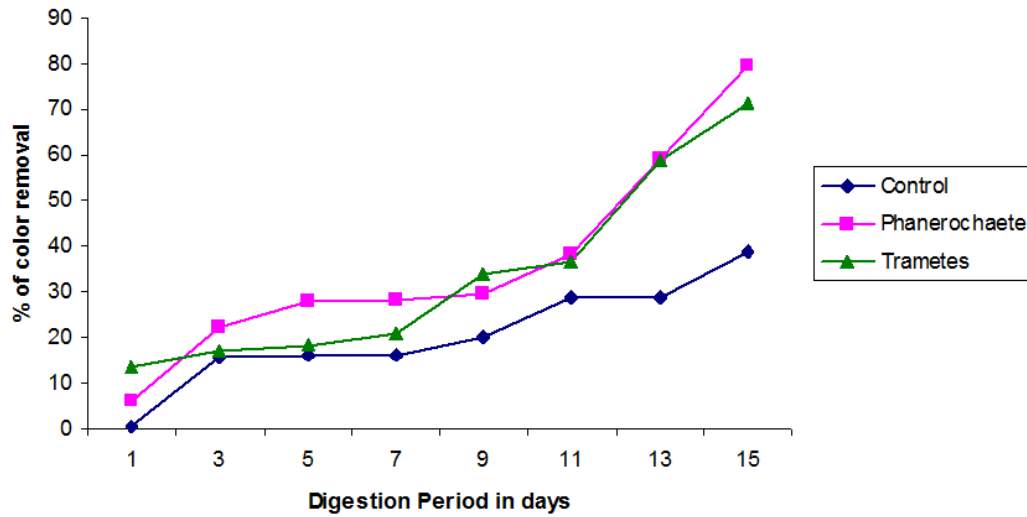
#### Determination of Co<sub>2</sub> release during biodegradation

When biodegradation is complete, the end products are mostly carbon-dioxide and water. Hence in the present study the rate of biodegradation is estimated in terms of carbon dioxide released. The seven-day-old fungal culture was added to the culture bottle containing soil samples. Total carbon dioxide released during degradation was measured<sup>4</sup>.

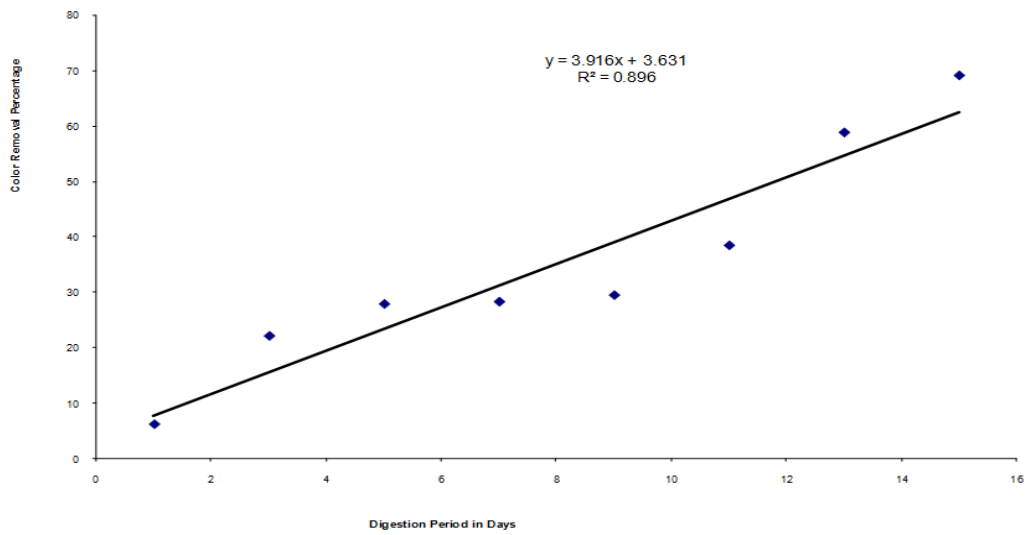
### RESULTS AND DISCUSSIONS

The Physico-chemical characteristics of dye effluent samples are presented in Graph 1, 2, 3 &4. From the observations it was clear that all the parameters reported were above permissible levels. The effluent pH ranged from 11-12. The amount of chlorides was maximum in effluent, which varied from 675mg/l. The alkalinity of effluent was in a range from 2450-3000 mg/l, metals like Zinc, Chromium (hexavalent) are 27 and 17mg/l reported respectively.

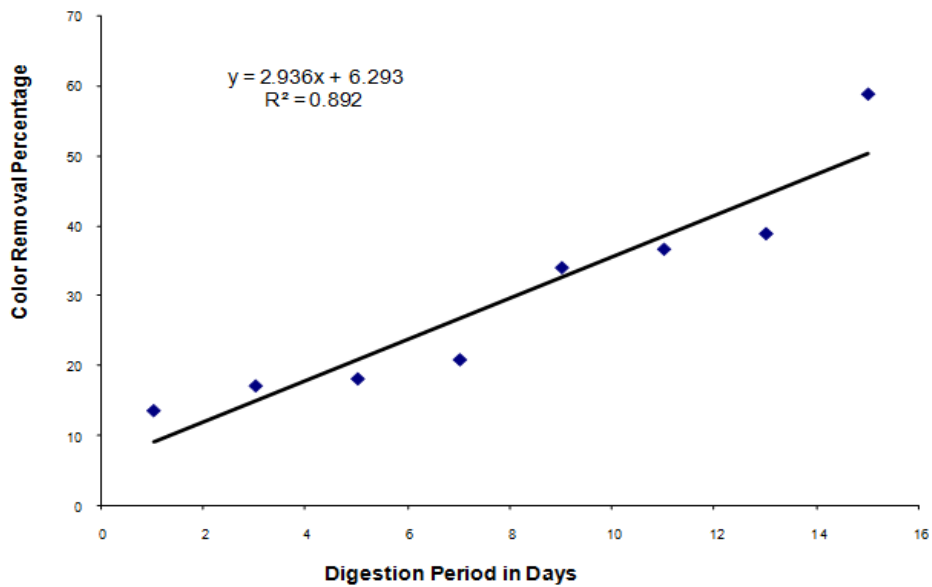
The 7<sup>th</sup> day cultures of *Phanerochaete chrysosporium* and 5<sup>th</sup> day cultures of *Trametes hirsuta* were chosen as test strains to avoid the initial lag phase and minimization of rate and length of exponential phase. The color removal, COD and BOD were analyzed initially after 24 hours of digestion of effluent with the microorganism. This time is required for the growth and adaptation by fungus to the dye effluent. In an optimum digestion time of 15 days, a maximum 79.51% of color reduction with hydraulic retention time (HRT) of 15days was observed. Maximum color reduction of 79.51% was observed with *Phanerochaete chrysosporium*, a white - rot fungi predominantly which has been recommended for the treatment of lignocellulose waste compared to *Trametes hirsuta*, which showed 71.51 % of decolorisation. From Graph - 1a one can predict that the decolorisation percentage was moderate in 7 days and maximum in 15 days of incubation. In view of the result obtained, one can conclude that the use of *Phanerochaete chrysosporium* species for decolorisation of azodye effluent is possible to clean up pollution load. Similar studies on decolorisation of dye contaminated soil and dye effluent by soil microorganisms are reported by Nikhath Kousar and Singara Charya<sup>5</sup> and Pointing, Bucher and Vrijmoed<sup>6</sup>. The colour reduction by fungal cultures had a highly significant correlation with the period of digestion of effluent (R<sup>2</sup> - 0.8964 & 0.8922 for *Phanerochaete chrysosporium* and *Trametes hirsuta* respectively) under static conditions (Graph 1b & 1c).



Graph 1a: Showing the percentage of Color removal of Dye Effluent by Test Fungi



Graph 1b: Showing the percentage of Color removal of Dye Effluent by Test Fungi (*Phanerochaete chrysosporium*)



Graph 1c: Showing the percentage of Color removal of Dye Effluent by Test Fungi (*Trametes hirsuta*)

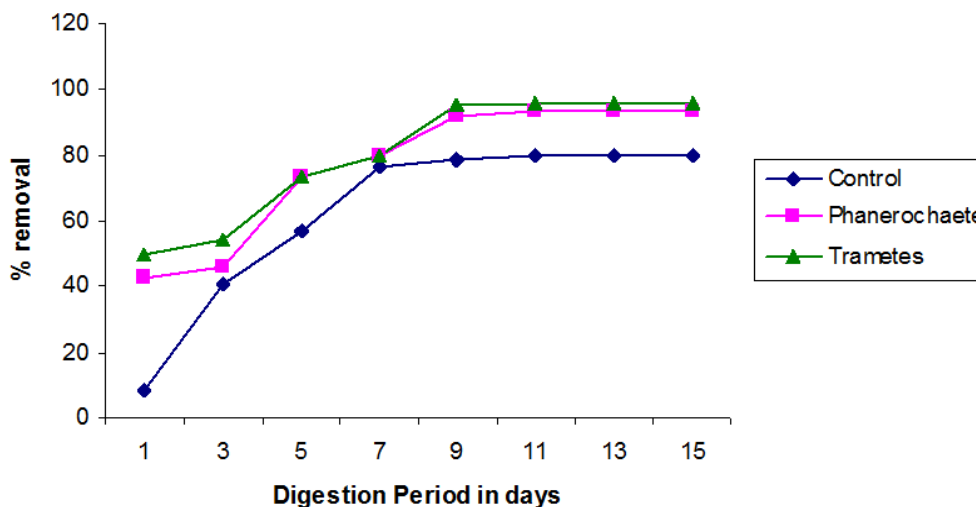
The observed value of pH was 12.09 on initial day, which decreased to 10.90 at 15 day, 12.0 to 9.05 and 11.90 to 8.90 for Control, *Phanerochaete chrysosporium* and *Trametes hirsuta* respectively. The value of pH was decreased due to the incubation of test fungal strains in effluent for their nourishment. Conductivity was found to decrease from 29.3 to 16.90, 28.7 to 12.90 and 28.0 to 12.30 for Control, *Phanerochaete chrysosporium* and *Trametes hirsuta* respectively. Turbidity change was from 4.5 on 1<sup>st</sup> day to 1.5 on 15<sup>th</sup> day for Control and from 3.2 to 0.5, 2.6 to 0.7 for *Phanerochaete chrysosporium* and *Trametes hirsuta*. The value of Alkalinity decreased from 2400mg/l on the 1<sup>st</sup> day to 1450mg/l on 15<sup>th</sup> day for Control and from 2000mg/l to 350mg/l, 1900mg/l to 300mg/l for *Phanerochaete chrysosporium* and *Trametes hirsuta* respectively.

A decreasing trend was observed in Total solids with 28,600mg/l on 1<sup>st</sup> day to 11,700mg/l on 15<sup>th</sup> day for Control and from 21,000 to 7,000mg/l and 28,200 to 6000mg/l for *Phanerochaete chrysosporium* and *Trametes hirsuta* respectively. The value of Total Dissolved solids decreased from 20,400 on 1<sup>st</sup> day to 8,600mg/l on 15<sup>th</sup> day for Control and from 19,800mg/l to 3500 and 18,000mg/l to 2500mg/l for *Phanerochaete chrysosporium* and *Trametes hirsuta* respectively. Suspended solids showed a decrease from 4,000mg/l on the 1<sup>st</sup> day to 3000mg/l on the 15<sup>th</sup> day for Control and 3800mg/l to 900mg/l and 3900mg/l to 1000 mg/l for *Phanerochaete chrysosporium* and *Trametes hirsuta* respectively.

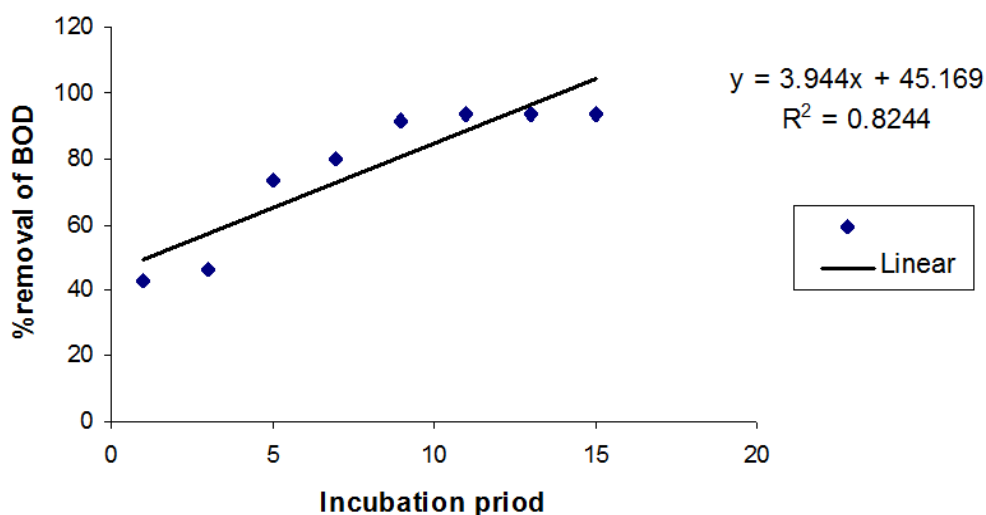
The observed value of Total Hardness was found to be 3,500 on initial day, which decreased to 1300 on 15<sup>th</sup> day and from 3900mg/l to 400mg/l and 1150mg/l to 150mg/l for *Phanerochaete chrysosporium* and *Trametes hirsuta* respectively. Chlorides and Nitrates are decreased from 645.17mg/l on 1<sup>st</sup> day to 322.59 on 15<sup>th</sup> day and from 2500mg/l to 2500mg/l respectively.

The effluent of inoculum digestion time on pollution abatement is present in Graph 2a, b, c & d. There is an increasing trend in BOD reduction with increase in digestion time. The 5-day-old *Trametes hirsuta* culture reduced maximum 96% with in 15 days of digestion where as 7-day-old *Phanerochaete chrysosporium* culture reduce BOD to 93.33%. The BOD reduction in effluent is highest (96.0%) by *Trametes hirsuta* (MTCC-136) (Graph -2a&b). *Phanerochaete chrysosporium* (MTCC-787) is capable of degrading a wide range of organo pollutants<sup>7</sup>. This fungus is able to degrade a wide range of dyes at faster rate<sup>8</sup>.

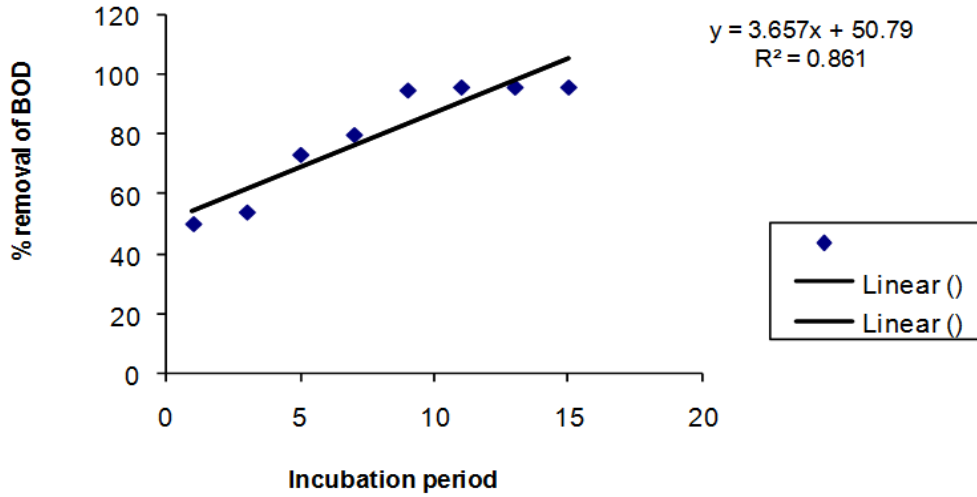
There is an increasing trend in COD reduction with increasing in digestion time. The 5day old *Trametes hirsuta* culture reduced COD to maximum 93.58% within 15 days of digestion, where as *Phanerochaete chrysosporium* reduced COD to 87.17% (Graph 3a&b). The percentage of BOD and COD reduction by fungal cultures was correlated with digestion period and showed a significant correlation ( $R^2 = 0.8244$  &  $0.8615$  (BOD for *Phanerochaete* & *Trametes* respectively) &  $R^2 = 0.9278$  &  $0.8031$  (COD for *Phanerochaete* & *Trametes* respectively)) under static conditions (Graph 2b&2c and 3b & 3c)



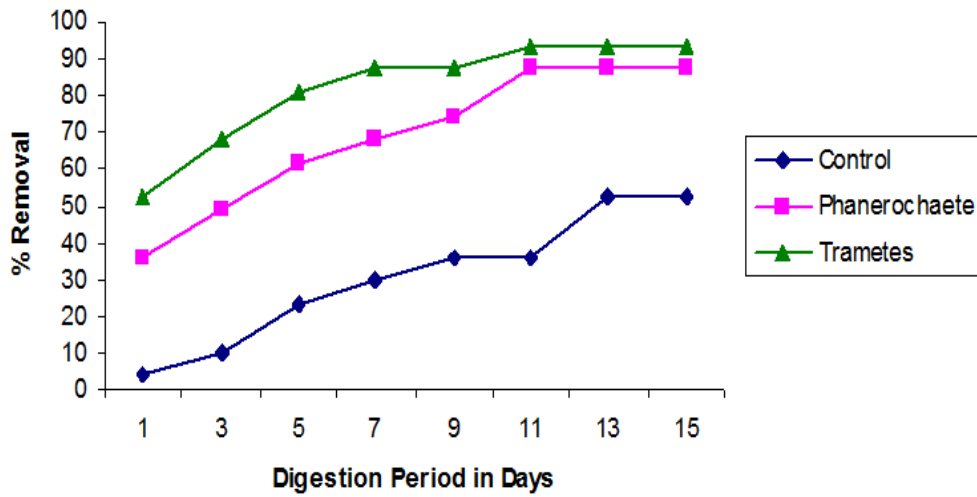
Graph 2a: Showing percent BOD removal of Dye Effluent by Fungal Cultures



Graph 2b: Showing percent BOD removal of Dye Effluent by Fungal Cultures (*Phanerochaete chrysosporium*)

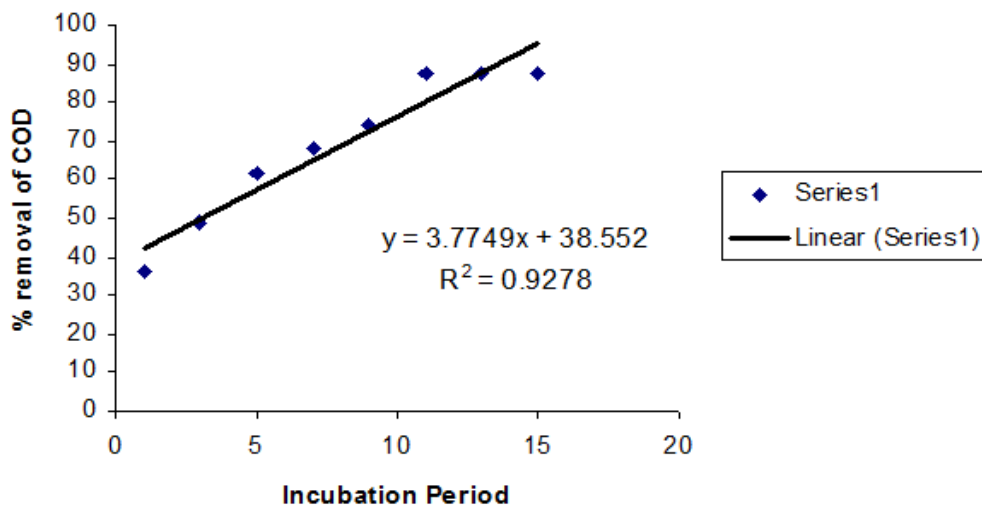


Graph 2c: Showing percent BOD removal of Dye Effluent by Fungal Cultures (*Trametes hirsuta*)

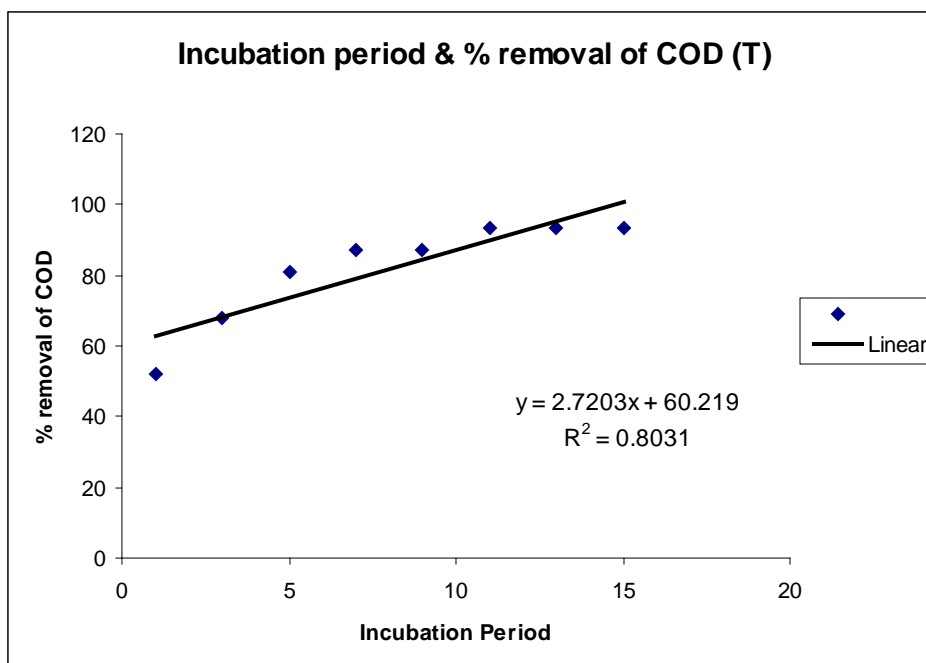


Graph 3a: Showing percent COD removal of Dye Effluent by Fungal Cultures

**Incubation Period & % Removal of COD (P)**



Graph 3b: Showing percent COD removal of Dye Effluent by Fungal Cultures (*Phanerochaete chrysosporium*)

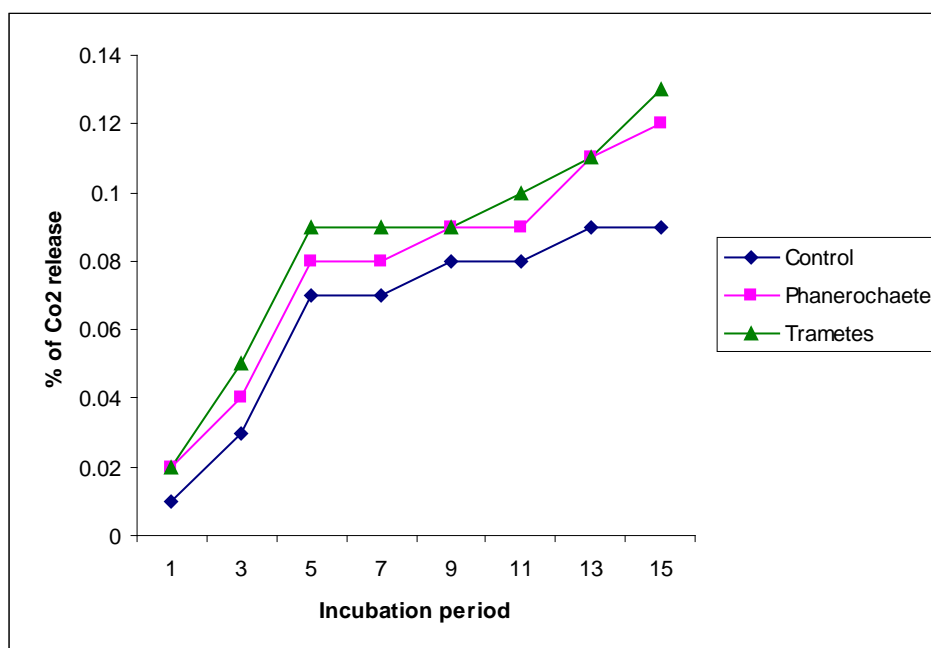


**Graph 3c: Showing percent COD removal of Dye Effluent by Fungal Cultures (*Trametes hirsuta*)**

The measurement of carbon dioxide release during the biodegradation process may be used as index of effluent degradation<sup>9</sup>. On the fifth day biodegradation CO<sub>2</sub> release by *Trametes hirsuta* showed a maximum of 8.36mg/ml and *Phanerochaete chrysosporium* showed 7.48mg/ml CO<sub>2</sub> release after 4 days of biodegradation (Graph 4). On the 11<sup>th</sup> day of biodegradation CO<sub>2</sub> *Trametes hirsuta* showed a maximum of 9.68mg/ml where *Phanerochaete chrysosporium* showed less amount of CO<sub>2</sub> release 9.24mg/ml. At the end of 15<sup>th</sup> day biodegradation the maximum total amount of CO<sub>2</sub> release 12.98mg/ml was found in *Trametes hirsuta*, followed by 12.54mg/ml with *Phanerochaete chrysosporium*. From this result it can be concluded that *Phanerochaete chrysosporium* and *Trametes hirsuta* strains are fast dye effluent bio-degrading fungi. This study reveals that the fungi like *Trametes hirsuta* and

*Phanerochaete chrysosporium* strains are capable of producing dye degrading enzymes at a faster rate to decompose dye substrate and release more CO<sub>2</sub> and hence these fungi can be utilized effectively as agents of biodegradation in waste treatment technologies.

The competition among the species was studied by the antagonism studies where in the growth of *Phanerochaete sp* in competition with *Trametes sp* was observed to be equally scattered in the petriplates which was same with *Trametes sp* in competition with *Phanerochaete sp*. This was also proved from the CO<sub>2</sub> release and Biodegradation of Dye effluent where in *Phanerochaete sp* was able to degrade effectively in competition with the indigenous species, which does not contain any antagonistic characters for *Phanerochaete sp*.



**Graph 4: Showing percent of CO<sub>2</sub> release after Biodegradation of Dye Effluent**

## CONCLUSION

The idea of exploiting fungi for abatement of pollution is not a novel one. It is now clear that fungal treatment of dye effluent with *Trametes hirsute* and *Phanerochaete chrysosporium* to mitigate organic pollution is an effective technique for eco - friendly pollution management. This microbial process brings down significantly the pollution load of dye effluent. Cost of treatment will be dependent on a number of factors, most important of which is the efficiency with which white - rot fungi will achieve effluent. The biodegradation of effluent with basidiomycetes once adopted itself to the system conditions (environment), the requirement of extra nutrients will certainly come down and the fungal process will become economical for commercialization. The present investigations elucidate that the aerobic digestion of the dye effluent can be carried out by subjecting it to treatment with *Trametes hirsuta* (MTCC-136) and white rot fungus *Phanerochaete chrysosporium* (MTCC - 787) under optimal cultural and process conditions.

## ACKNOWLEDGEMENT

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