

## Mycoremediation of tannery effluent using spent bags of edible mushroom *Calocybe indica*

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### Abstract

Bioremediation of tannery effluent using spent bags of milky mushroom mycelium *Calocybe indica* was undertaken to effectively utilize the discarded bags after harvesting the edible mushroom. Mycelium grown in rice straw was used for effluent treatment. The rate of bioremediation was enhanced by adding co-metabolites like carbon, nitrogen, phosphorous source in the form of carbon as glucose and sucrose; nitrogen as urea and ammonium chloride; phosphorus as ortho phosphoric acid. After treatment, effluents were analysed by spectrophotometer. Bioassay of treated effluents were done by germinating green gram seeds; to study the suitability of the effluent for irrigation and to find out the toxic effect of the treated effluent on seed germination. For the bioassay shoot and root length of germinated green grams were measured. The result, prove that the addition of nitrogen and phosphorous source enhance myremediation of tannery effluent. Using Milky Mushroom mycelium to treat the tannery effluent proves to be an efficient and ecofriendly venture.

**Keywords:** tannery effluent, *calocybe indica*, co-metabolism, bioassay, glucose, phosphorous nitrogen

### Introduction

Environmental pollution is one of the major problems of the world and is increasing in an exponential rate due to urbanization and industrialization. Industrial processes lead to the production of large amount of toxic and stable pollutants, which are all collected into the water coming out from the plant. The disposal of these contaminated effluents into receiving waters can cause environmental damage, directly influencing the aquatic ecosystem and in turn human beings (Prigione, et. al. 2008). One of the primary roles of fungi in the ecosystem is to decompose, performed by mycelium. The mycelium secretes extracellular enzymes and acids that breakdown lignin and cellulose, the two main building blocks of plant fiber (Goltapeh *et al.*, 2013) [13].

The important process of removal of metals/pollutants from the environment by mushroom is - *biosorption*. Biosorption is considered as an alternative to the remediation of industrial effluents as well as the recovery of metals present in effluent. Biosorption is a process based on the sorption of metallic ions/pollutants/xenobiotics from effluent by live or dried biomass which often exhibits a marked tolerance towards metals and other adverse conditions (Gavrilescu, 2004) [7]. Biosorbents can be prepared from mushroom mycelium and spent mushroom compost. Aalapti Singh and Pammi Gauba (2014) [1] reported the significant role of fungi in bioaccumulation of heavy metals from soil contaminated with heavy metals.

Tannery effluent plays a major role in environmental pollution in developing countries like India. The increase in untreated or partially treated wastewater and industrial effluents into natural ecosystems pose a serious problem to the environment (Aravindhan *et al.*, 2007) [3]. Traditional technologies for metal removal include complexation and precipitation,

filtration, reverse osmosis, adsorption, etc. (Vieira *et al.*, 2011). Bioremediation is one of the most promising technologies for treating industrial wastes, municipal or urban wastes, mining wastes (including effluents containing heavy metals, etc). It can be used as in situ remediation technology with indigenous microorganisms or can be used in ex situ mode in either composite piles or bioreactors (Ranensan and Chakrabarti, 2009) [5]. Bioremediation is considered as cost effective and eco-friendly technology for decontamination sites which are highly contaminated with pollutants. The process of bioremediation uses various agents such as bacteria, yeast, fungi, algae and higher plants as major tools in treating pollutants in the environment.

Mycoremediation is a form of bioremediation in which fungi are used to decontaminate the area. The key to mycoremediation is determining the right fungi species to target a specific contaminant (Danesh *et al.*, 2013) [12]. Some mushroom species are known to degrade several of these, while others are more selective. Several Fungi are the chief agents for the degradation of environmental pollutants. A plot of soil contaminated with diesel oil was inoculated with mycelia of *Pleurotus* in traditional bioremediation techniques and after four weeks, more than 95% of the Polycyclic aromatic hydrocarbons reduced to non-toxic components in the mycelia-inoculated plots. A number of basidiomycetes were reported to have great promise for heavy metal ions removal from waste water since their mycelium excretes enzymes that breakdown complex substances into simpler molecules and absorb heavy metals (Gadd, 2000) [6]. In this study milky mushroom mycelium from harvested bags were used for treatment of tannery effluent. The rate of mycoremediation was enhanced with addition of sugar, nitrogen and phosphorous sources.

## Materials and Methods

**Collection of Samples:** The effluent was collected from Tannery industries in 10 liters clean can. It was maintained in room temperature. Cultivated and harvested bags of Milky Mushrooms were collected from Indis milky mushrooms, Karumpukuppam, Ambetkar Nagar, New Gummidipoondi, Thiruvallur – 601201. Fungal mycelium along with the substratum rice straw was used for treatment.

**Mycoremediation of Tannery Effluent by Spent Bag of Calocybe Indica:** Untreated effluents (100 ml) was poured in to enamel tub to which co-metabolite source like carbon, nitrogen, and phosphorous were added in the concentration of 1mM. To this 10 g of fungal mycelium along with rice straw was inoculated. Set up was maintained for seven days. After seven days, the treated effluent was filtered with muslin cloth. The wet weight and dry weight of the fungal mycelium were determined and tabulated.

The absorbancy of the treated effluent was analyzed at 660nm in UV-VISIBLE Hitachi Spectrophotometer.

**Bioassay:** Filtered treated effluents were used to germinate

green grams seeds. Root and shoot length were measured and results were tabulated.

## Result and Discussion

The study was designed in order to understand the efficiency of the discarded bags of *Calocybe indica* to remediate tannery effluent. In this view fungal mycelium along with rice straw were added to tannery effluent along with various co-metabolites. After seven days, the effluent were filtered and the filtrate were analyses for UV-Visible spectrophotometer. The effect of treated effluent on the growth of root and stem were observed.

## Growth Analysis

The growth rate of *Calocybe indica* was studied in response to tannery effluent. Simultaneously the bioremediation efficiency of the fungi was studied. The mycelium was grown in tannery effluent. The effect of additional sources of nutrients on the rate of bioremediation proves to be effective. Both phosphorous and nitrogen source enhance the bioremediation efficiency as observed from the growth rate of the fungus (Table 1) and the spectral readings (Graph 1).

**Table 1:** Growth rate of *Calocybe indica* in effluent in response to additional carbon, nitrogen and phosphorous source.

S. No	Samples	Initial weight(g)	Wet weight (g)	Dry weight (g)
1.	Raw effluent	10	12.05	5.73
2.	Glucose	10	10.21	3.98
3.	Sucrose	10	9.69	5.34
4.	H <sub>3</sub> PO <sub>4</sub>	10	16.57	6.73
5.	NH <sub>4</sub> Cl	10	12.26	5.98
6.	Urea	10	10.87	5.70

## UV – Visible Spectrophotometry Reading

One of the effluent was read at 660nm and water as blank. The filtrates of fungal mycelium was also subjected to spectrophotometer analysis at 660nm. The reading were noted and represented on graph. (Graph.1)

## Bioassay

The effect of treated effluent on the germination of green gram was analysed (Table 2). The germination of seeds were observed on 3 day, shoot length and root length were measured and tabulated.

**Table 2:** Bioassay of treated effluent using green gram seed.

S. No	Samples	No. Of Seeds	No. of Seeds Germinated	Germination Percentage	Root Length (Cm)	Shoot Length(Cm)
1.	Control (Water)	10	9	90	10.9	2.7
2.	Raw Effluent	10	5	50	1.6	-
3.	Treted Raw Effluent	10	8	80	1.9	-
4.	Glucose	10	2	20	.3	-
5.	Sucrose	10	6	60	1.1	-
6.	H <sub>3</sub> PO <sub>4</sub>	10	8	80	2.5	-
7.	NH <sub>4</sub> CL	10	8	80	2.6	-
8.	UREA	10	3	30	.8	-

In present study, Mycoremediation of tannery effluent by *Calocybe indica* is enhanced in presence of Nitrogen and Phosphorous as co-metabolites source, as observed from the reading OD of the treated effluent. *Calocybe indica* has the biosorption efficiency of heavy metals at highest applied concentration (Surumbar Kuzhali, *et al.*, 2012) <sup>[8]</sup>.

It is proved that mushrooms have different abilities of biosorption, bioremediation, bio degradation and toxicity reduction. In my opinion, researchers should try to first remediate the heavy metals by cultivating high metal

absorbing species of mushroom. Researchers should also try to develop the method of using biomass repeatedly for the biosorption of pollutants which will also reduce the waste generation. Immobilized cells and mycelium proves to be effective in bioremediation of tannery effluent (Kamalashankari, 2010; Devi, 2011) <sup>[10]</sup>.

## Conclusion

Mushroom is a tremendous boon to the idea of using this for mycoremediation process as a real-world solution. The

cultivation of edible mushroom on agricultural and industrial wastes may thus be a value added process capable of converting these discharges, which are otherwise considered to be wastes, into foods and feeds. Besides producing nutritious mushroom, it reduces genotoxicity and toxicity of mushroom species. Mycoremediation through mushroom cultivation will alleviate two of the world's major problems i.e. waste accumulation and production of proteinaceous food simultaneously. Thus, there is a need for further research towards the exploitation of potential of mushroom as bioremediation tool and its safety aspects for consumption as product.

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