

Remediation of Wastewater using Mushroom: *Pleurotus ostreatus* (Oyster Gill Mushroom)

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Abstract-Globally, the water pollution is one of the most common problems of the world. The water pollution increases day by day due to increasing urbanisation, industrialisation, population growth etc. and it affects the ecosystem. The degradation of environment results in adverse effect on living organisms and agriculture. The goal of the study is to remediate the wastewater by mushroom: *Pleurotus ostreatus* by checking its physico-chemical parameters before and after the treatment.

Mushrooms are produced on natural materials taken from agriculture wastes like wheat straw, rice straw, etc. Mushroom has been used as a product for consumption since a long time due to their richness in protein. Mushrooms are also known as mycoremediation tool because of their use in remediation of different types of pollutants. Mycofiltration is the process of using fungal mycelium as an active biological filter for removing pollutants from wastewater.

Keywords- Mushroom, Mycoremediation, Mycelium, Mycofiltration, *Pleurotus ostreatus*

1. INTRODUCTION

Mushrooms are the fleshy fungi, which constitute a major group of lower plant kingdom. The mushroom is a common fungal fruiting body that produces basidiospores at the tip of club like structures, called basidia, which are arranged along the gills of the mushroom.

Water is the universal solvent and is one of the important natural resource. In recent years, many of the water bodies have become polluted by sewage, industrial wastes and synthetic chemicals (Hariharan and Varshya, 2002).

Water pollution is the prime cause of unavailability of suitable water for irrigation purposes. High level of pollutants mainly organic matter in river water causes an increase in biological oxygen demand, chemical oxygen demand, total dissolved solids, etc. They make water unfit for drinking, irrigation or

any other use. When the effluent is released into the environment without the proper treatment, it alters the characters of the ecosystem. Farmers are using these raw effluents for irrigation and found that growth, yield and soil health are reduced (Nandy and Kaul, 1994). Water is the basic and primary need for all vital life processes. With increasing industrialization and population growth water sources available for various purposes such as drinking, recreation, aquaculture, agriculture have been adulterated with industrial as well as animal and human wastes. Polluted sewage water contains solid and dissolved organic compounds that imparts an offensive colour and serve as an excellent medium for the growth and multiplication of microorganisms.

Mycoremediation is a pollution control technology that uses biological system to

catalyse the degradation or transformation of various toxic chemicals to less toxic forms. Mycoremediation could be employed for the treatment of various industrial effluents including sewage water, dairy industrial sludge, effluents

from tannery, paper and pulp industry. Mycoremediation is cost effective and efficient method of decontamination that has become increasingly popular now a day to remove environmental population.

2. MATERIALS AND METHODS

2.1 Study area

The sewage treatment plant (STP) of Dayalbagh, Agra discharged its water after the chlorination process in the fields for irrigation purposes. But, this water is not as much suitable for irrigation because foams are occur in the water due to presence of biodegradable surfactants (e.g. household detergents) from industrial and municipal waste water, excess production of extracellular polymeric substances (EPS) by activated sludge under nutrient condition. So, our aim of study is to check the physico-chemical parameters of the STP water and then remediate it by mycoremediation to make the water suitable for agricultural purposes. In our present study, we remediate the waste water of STP by two methods: Firstly, by Mushroom Extract (M.E.) Secondly, by Mycofiltration.

2.2 Collection of wastewater sample

The waste water samples were collected from the mounds of the fields in which STP water get discharged for irrigation purposes which are located in the Department of Botany, D.E.I., Dayalbagh, Agra. These samples were used for testing the physico-chemical parameters and for the remediation of wastewater by mushroom extract and by mycofiltration in *invitro* conditions.

2.3 To check the initial and final physico-chemical parameters (Colour, odour, TDS, DO, BOD, COD, Hardness, Acidity, and Alkalinity) of collected wastewater

The analysis of physico-chemical parameters of treated and untreated water samples would be carried out by Ambasht (1990) and Aneja (2001).

2.4 Collection of Mushroom strains

The experiment was conducted in the Microbiology laboratory, Department of Botany, D.E.I., Dayalbagh, Agra. The pure cultures of spawn were obtained from Solan, Himachal Pradesh.

2.5 Spawn preparation

Spawn is a fungal growth medium infused with mycelial fragments of mushroom which serves as inoculum for mushroom cultivation. There is a great problem in preparing pure spawn of a particular strain of a mushroom because of fungal, bacterial or viral contamination.

The steps of grain spawn (e.g. rye/sorghum/wheat grains) or straw spawn (paddy/wheat straw) preparations follow –

- (a) Cooking the grains in water until they swell/cutting of straw into

5cm long pieces and soaking in water for 5-10 minutes.

- (b) Decantation of water, mixing of 20% lime (calcium carbonate)
- (c) Transferring into glass tubes/flasks.
- (d) Plugging with cotton.
- (e) Autoclaving at 121°C for 30 minutes and cooling down to $30-45^{\circ}\text{C}$.
- (f) Inoculating the substrate with pure culture of mushroom.
- (g) Incubation at suitable temperature for proper infestation of mycelium for their use as spawn (Change and Li, 1982).

2.6 Cultivation of Mushroom

2.6.1 Cultivation of Oyster Mushroom (*Pleurotus ostreatus*)

The spawns of *Pleurotus ostreatus* are stored at $20-25^{\circ}\text{C}$ temperature in pre-sterilised area i.e. in B.O.D incubator.

2.6.2 Requirements

- Thatched hut/ polyethylene chamber, mud/ pucca house.
- Formaldehyde solution.
- Dry paddy straw (chopped) or other agro-wastes.
- Bavistin (fungicide).
- Spawn of *Pleurotus* spp.
- Polythene bags.
- Water sprayer.

2.6.3 Procedure

- Take dried chopped straw in the large tub.
- Soak the chopped straw into water and,
- Add Bavistin powder.
- Add Formalin solution (50ml for 10 kg wheat straw).

- Wheat straw was soaked in water containing bavistin and formaldehyde solution for 24 hours.
- Drain off the excess water.
- Dry it in the sunlight until little moisture is left.
- Add spawns.
- Mix all the constituents.
- Fill the mixture into polythene bags with holes
- Keep the field bags in a room at 21 to 30°C with sufficient light and ventilation for 15-16 days for spawn running.
- Cut open the polythene bags on the sides without disturbing the bed.
- Spray water over the bags daily.

2.6.4 Observation

Observe for the mushroom crop after 3-4 days of opening the polythene bags (i.e. after 20-22 days of spawning).

Harvesting: First harvesting to be done 20-22 days after spawning, second harvesting 27-29 days after spawning and third harvesting 34-36 days after spawning.

2.6.5 Precautions

- For better yields, temperature of the room should be in the range of $20-26^{\circ}\text{C}$ and relative humidity of 70-90%
- Polythene bags with holes should be used.
- Spray the water with water sprayer at regular interval of time.

2.7 To check the chemical toxicity of cultivated mushroom

A drop of juice was pressed out of the fresh body of the mushroom on a piece of paper. After the spot had dried hydrochloric acid (HCl) was dropped on it. A blue spot indicate presence of toxins. After the juice is dried, we add a drop of HCl on it and then a blue spot doesn't appear. Thus, it indicated that there are not any toxins in the cultivated mushroom. So, we can say that it is edible in nature.

2.8 Remediation of wastewater by Mushroom Extract and by Mycofiltration

2.8.1 To prepare the extract of cultivated mushroom

After plucking the fruiting bodies, they are dry in oven at 50°C. Make the powder of the dried fruiting bodies by the grinder. The extract is prepared through soxhlet extraction using 95% methanol as solvent. The filtered extract was kept in sterilized flasks or in glass vials and kept in refrigerator at 4°C for further use. Soxhlet extraction is one of the oldest techniques for isolating metabolites from natural material. The technique is used for isolation and enrichment of analytes of medium and low volatility and thermal stability.

2.8.2 Remediation of wastewater by Mycofiltration

Mycofiltration is the name Paul Stamets gives to the “use of fungi to degrade or remove toxins from the environment” by using mycelial mats. Fungi can be used to clean up mercury,

polychlorobiphenols(PCBs), fertilizers, munitions, dyes, estrogen-based pharmaceuticals, neurotoxins including DDT dioxins, and stored nerve gas.

Mycofiltration is the use of mycelium as a membrane for filtering out micro-organisms, pollutants, and silt. Mycelium is the underground vegetative growth of a fungus needs a moist environment to thrive and produce mushroom. Mycelium forms this thread like network of interlacing cells that weave within one another, this network, web, bed whatever we like to call it catches particles and in some cases digest them. As the substrate (a supporting material on which mycelium can grow) debris is digested, microcavities form and fill with air or water providing buoyant, aerobic infrastructures with the vast surface area. The antibiotics and natural enzymes the Mycelium produces may not digest all of the pathogens that may be in the soil, but some bacteria such as *Bacillus subtilis* are blocked from reproducing and are forced into dormancy.

2.9 Analysis of physico-chemical parameters of untreated water, treated by mushroom extract and by mycofiltration

Physico-chemical parameters study is very important to get an exact idea about the quality of water and we can compare the results of untreated wastewater with treated water. The results of our present study were shown in tables and graph as given below:

3. RESULTS

3.1 Table: 1 Physico-chemical parameters of untreated water
Table: 2 Physico-chemical parameters of treated water by Mushroom Extract (M.E.)

Sample: Untreated	August	September	October	November
Color	Light Brown	Light Brown	Light Brown	Light Brown
Odour	Foul Smell	Foul Smell	Foul Smell	Foul Smell
pH	9.1	8.7	8.6	8.5
Acidity (mg/l)	325	402	495	462.5
Alkalinity (mg/l)	970	955	865	872
Hardness (mg/l)	758	756	713	698
TDS(mg/l)	1360	1410	1240	1250
DO(mg/l)	3.8	2.5	4	2.4
BOD(mg/l)	29.9	18.6	38.2	16.8
COD(mg/l)	152.9	168	144	186.8

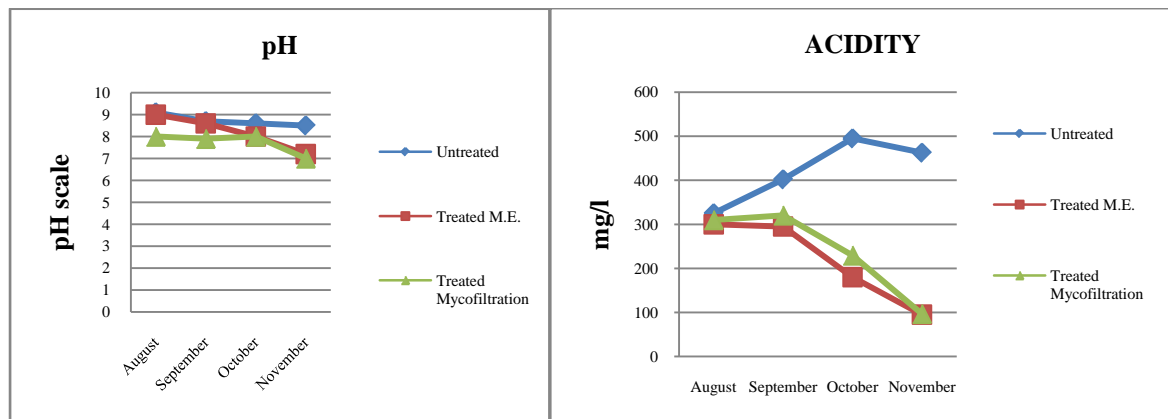
Sample: Treated M.E. (Mushroom Extract)	August (5ml M.E. in 250 ml untreated water)	September (10 ml M.E. in 250 ml untreated water)	October (15ml M.E. in 250 ml untreated water)	November (20 ml M.E. in 250 ml untreated water)
Color	Light Yellow	Light Yellow	Light Yellow	Light Yellow
Odour	Odourless	Odourless	Odourless	Odourless
pH	9	8.6	8	7.2
Acidity (mg/l)	300	295	180	95
Alkalinity (mg/l)	860	752	610	571
Hardness (mg/l)	610	608	550	510
TDS(mg/l)	1150	980	870	520
DO(mg/l)	1.2	2.8	3.7	4.9
BOD(mg/l)	3.6	17.9	14	9.6
COD(mg/l)	150.3	148.2	102	78

Table: 3 Physico-chemical parameters of treated water by Mycofiltration

Treated Myco-filtration	August	September	October	November
Color	Dark Yellow	Dark Yellow	Dark Yellow	Dark Yellow
Odour	Odorless	Odorless	Odorless	Odorless
pH	8	7.9	8	7
Acidity (mg/l)	310	320.5	229.6	97
Alkalinity (mg/l)	615	600.3	615.7	558
Hardness (mg/l)	710	678	634	610
TDS (mg/l)	1270	1185	1190	1165
DO (mg/l)	4.3	3.7	3.9	3.8
BOD (mg/l)	15.6	28.3	23.2	28.2
COD (mg/l)	55.7	43.2	23.2	28.2

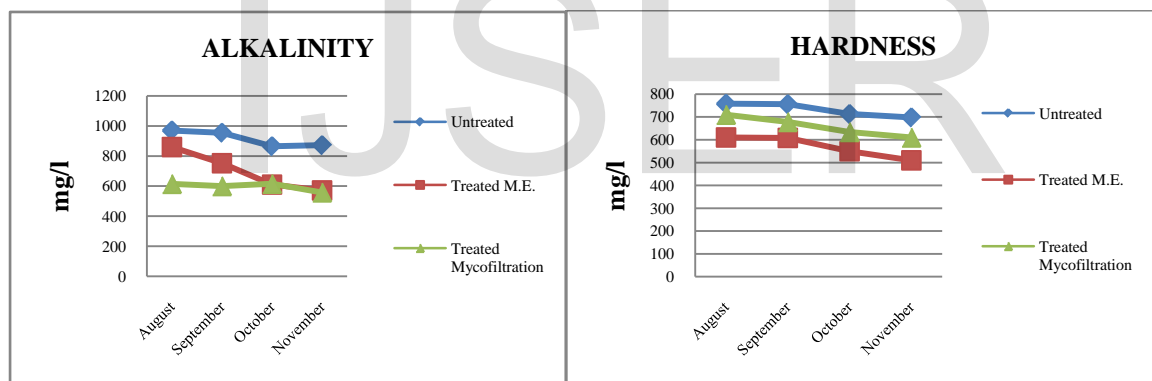
3.2 Graphical representation of the physico-chemical parameters of untreated water, treated by mushroom extract and by mycofiltration

Graph 1 Graph 2



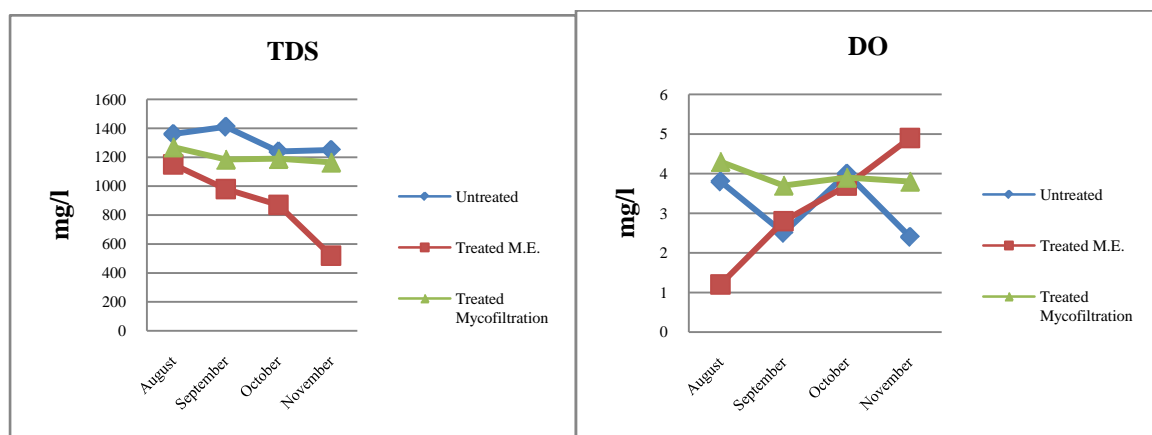
Graph 3

Graph 4

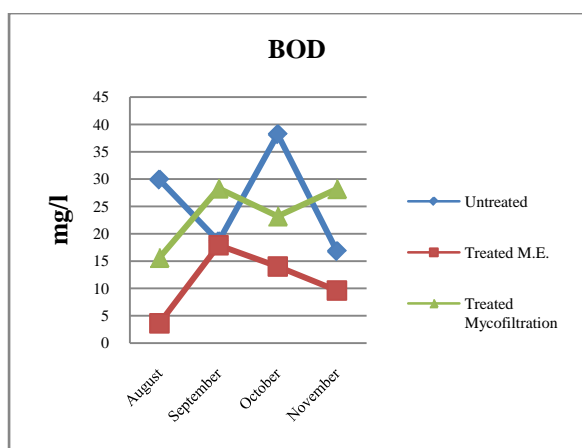


Graph 5

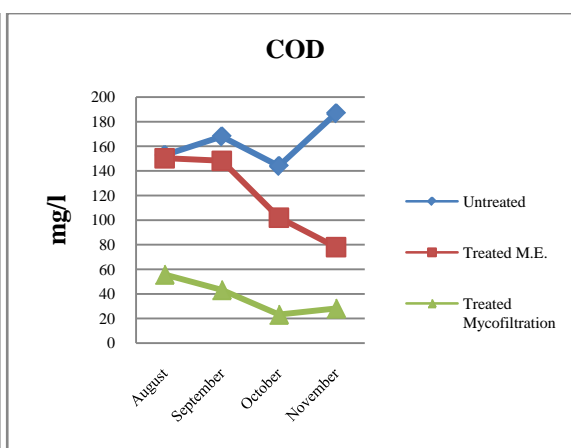
Graph 6



Graph 7



Graph 8



4. DISCUSSION

Ever since the origin of man, the human life covering the entire spectrum from basic essentials of life, food, water and shelter i.e. from the settlement to the fundamentals of civilization including the socio-economic, cultural, religious aspect are invariably associated with water. This has led to the use and misuse of water and due to this water becomes polluted. In the present investigation the sample were collected from STP site. Physico-chemical parameters of untreated water were carried out. The remediation of water showed different values of pH, acidity (mg/l), alkalinity (mg/l), hardness (mg/l), TDS (mg/l), DO (mg/l), BOD (mg/l), and COD (mg/l). In the present study, we attempted two investigational systems: one is remediation of wastewater by the mushroom extract and the other is remediation of wastewater by mycofiltration. After the treatment, the physico-chemical parameters were checked by comparing it with the physico-chemical parameters of untreated water as shown in the graphs.

Results of the physico-chemical parameters of untreated water are given in Table: 1. The physico-chemical parameters

measured which were colour, odour, pH, acidity, alkalinity, hardness, Total Dissolved Solids (TDS), Dissolved Oxygen (D.O), Biological Oxygen Demand (B.O.D), and Chemical Oxygen Demand (C.O.D).

Table: 1 depicted the physico-parameters of wastewater samples and observation was taken with the subsequent interval of 4 months. The colour and odour of the samples of STP site with an interval of 4 months were the same. The colour of the water observed at the site was light brown with foul odour. Therefore, wastewater can reduce the solubility of oxygen and amplified odour due to anaerobic reaction (Akan et al.2009). From the data collected from our present study, the physico-chemical parameters showed high values as compared to their permissible limits. The pH of the untreated water of four months from August, September, October, and November was observed indicate the waste water was highly alkaline i.e. 9.1, 8.7, 8.6, and 8.5 respectively. The Acidity of the untreated water of four months from August, September, October, and November was 325mg/l, 402mg/l, 495

mg/l, and 462.5mg/l. The Alkalinity of the untreated water of four months from August, September, October, and November was 970 mg/l, 955 mg/l, 865 mg/l, and 872mg/l. The Hardness of the untreated water of four months from August, September, October, and November was 758 mg/l, 756 mg/l, 713 mg/l, and 698mg/l. The TDS of the untreated water of four months from August, September, October, and November was 1360 mg/l, 1410 mg/l, 1240 mg/l, and 1250 mg/l. The DO of the untreated water of four months from August, September, October, and November was 3.8 mg/l, 2.5 mg/l, 4 mg/l, and 2.4 mg/l. The BOD of the untreated water of four months from August, September, October, and November is 29.9 mg/l, 18.6 mg/l, 38.2 mg/l, and 16.8 mg/l. The COD of the untreated water of four months from August, September, October, and November was 152.9 mg/l, 168 mg/l, 144 mg/l, and 186.8 mg/l.

Aftab Begum et.al (2005) studied various physico-chemical parameters and analysis of untreated fertiliser effluent. His results revealed that parameters like TDS, DO, BOD, COD, are higher as compared to their permissible limits of Central Pollution Control Board (1995).

DeyKallul et.al (2005) studied various physico-chemical parameters of the sample of STP water and his results shows that the parameters like pH, Acidity, Alkalinity, Hardness, TDS, DO, BOD, COD were high as compared to the permissible limits.

Table: 2 represents the values of the water which was treated by mushroom extract of different concentrations in different months i.e. in August, 5ml of mushroom

extract was used in the 250ml of untreated water and then place it in BOD incubator for 3 days at 20⁰C. After 3 days, its physico-chemical parameters like pH, acidity, alkalinity, hardness, TDS, DO, BOD, COD were checked. There were only slight changes observed in the values of the physico-chemical parameters. Thus, concentration of extract was increased for optimization.

In September, 10ml of mushroom extract was used in the 250ml of untreated water and then place it in BOD incubator for 3 days at 20⁰C. After 3 days, its physico-chemical parameters like pH, acidity, alkalinity, hardness, TDS, DO, BOD, COD were checked. Simultaneously, in October 15ml of mushroom extract in 250ml of untreated water was used and place it in BOD incubator for 3 days at 20⁰C. After 3 days, its physico-chemical parameters like pH, acidity, alkalinity, hardness, TDS, DO, BOD, COD were checked. In November, 20ml of mushroom extract in 250ml of untreated water was used and place it in BOD incubator for 3 days at 20⁰C. After 3 days, its physico-chemical parameters like pH, acidity, alkalinity, hardness, TDS, DO, BOD, COD were checked. The best results were seen in November. Thus, it is clear from the present study that by increasing the concentration of the extract, the water becomes treated. Table: 3represents the values of the water, which were treated by mycofiltration in different months i.e. August, September, October, November. From the present study, the values of the physico-chemical parameters of the four different months are shown in the table.

Mycofiltration, a very similar process to mycoremediation, is the use of mycelial mats to filter toxic waste and microorganisms from polluted water.

Mycofiltration membranes have been applied to filter pathogens, including protozoa, silt, chemical toxins, bacteria, and viruses. (Stamets P., 2005).

Mycelium forms the thread like network of interlacing cells that weave within one another. As the substrate (a supporting material on which mycelium can grow) debris is digested, micro cavities form and fill with air or water providing buoyant, aerobic infrastructures with vast surface area. The antibiotics and natural enzymes, the mycelium produces may not digest all of the pathogens that may be in the soil, but some bacteria such as *Bacillus subtilis* were blocked from reproducing and were forced into dormancy. Lovy and others (1999) found that Paul Stamets polypore strain called zhu ling (*Polyporus umbellatus*), was 100% effective in inhibiting the malaria parasite.

5. SIGNIFICANCE

One of the major environmental problems faced by the world today is the contamination of water by toxic chemicals. Mycoremediation system depends upon microorganisms (fungi) native to contaminated sites. There are some examples of fungi, which are used in mycoremediation process are- *Pleurotus ostreatus*, *Agaricus bisporous*, *Phanerochaete chrysosporium*, *Lentinus edodes*, etc.

Mostly, all the populations directly dumped their waste products into the rivers and these wastes were also dumped in the STP. Due to the lack of water, the STP water was directly discharged into the fields for irrigation purposes without any barrier. Microorganisms as bacteria, fungi, etc. are present abundantly in the water

The potential of mycofiltration media to remove *E. coli* from synthetic storm water under laboratory conditions (Beutel and others 2014). Water contamination can be improved by the application of mushroom mycelial technologies. For example, the use of bioconversion processes to transform the polluting substances into valuable foodstuffs, e.g., the proper treatment and reutilization of spent substrates/composts in order to eliminate water pollution problems (Beyer, 2005, Noble, 2005).

In our present study, by using the mushroom extract and by mycofiltration of untreated water, the water becomes treated and it became suitable for irrigation purposes. Therefore, we can say that *Pleurotus ostreatus* is a powerful tool for the remediation of wastewater.

that makes it unfit for irrigation purposes. But, when we use fungi like *Pleurotus ostreatus*, for remediation of water, then fungi create an inhibitory effect of the growth of bacteria that are present in untreated water and this can be done by using a technique known as mycofiltration. Mycofiltration is a powerful and effective tool to remediate wastewater. In fact, it can play a pivotal role in breaking down toxic substances that are present in wastewater because *Pleurotus ostreatus* (oyster mushroom) are known to produce extracellular enzymes such as manganese peroxidase, laccase, lignin peroxidase and aryl alcohol oxidase, which are capable of degrading lignocellulosic biomass. So, Water purification technology is often complicated and requires sophisticated equipment. It is also expensive to run and

maintain. The Mycofiltration technology could prove a simple answer to the problem. It could be used to remove the toxic chemicals, bacteria, viruses, and other hazardous materials from water much more effectively at a lower cost than other conventional methods. Thus, by creating a barrier of mycelium mats of *Pleurotus ostreatus*, the wastewater gets suitable for irrigation purposes. There are

6. CONCLUSION

Pleurotus ostreatus can be used as a remediating tool to reduce pollutants from wastewater by the action of various extracellular enzymes such as lignin peroxidase, laccase, manganese peroxidase, which have the ability of degrading lignocellulosic biomass. Various researches have shown that mushrooms have the potential to remediate the wastewater. Mycoremediation is not a miracle, but it is

numerous advantages of using mycoremediation over commercialised technologies like public acceptance, nature and environment friendly, low cost, flexibility, fast, safety, simple, and quiet, low maintenance etc. It is eco-friendly and cost-effective and cheapest technology for the treatment of wastewater.

a powerful tool to remediate wastewater. It can play a crucial role in breaking down various toxic substances and helps in cleaning the environment. More research needs to be carried out on the potential of mushroom and its role in mycoremediation.

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