

Original Paper

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## **Laboratory Study of ‘Pharmaceutical Waste Management’ by Biological Treatment**

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### **Abstract**

Gujarat contributes about 40% of pharmaceutical sector revenues the formulation and production units in the out cuts of Ahmedabad are responsible for huge quantity of export and locally used pharmaceuticals. In the processes they generate waste and many of them try to manage it, but they require norms are not attain by all. In this work we have tried the advance treatment at laboratory scale to treat the liquid waste of pharmaceuticals to a level where norms can be attain. The procedures adopted were biological treatment with safety and ease of treatment. COD is an established parameter of pollution. These criteria have been recognized globally as an index of pollution level and treatability as well. COD was broad down from very high to the safe level by our microbial treatment. Three different fungal cultures are used for treatment of pharmaceutical waste water and they are efficient to bring down the COD of waste water. Cultures are grown in agar as well in broth, growth inoculated in the prepared system and incubated for few days for the growth of the fungi. It has been supported with the evidences of physicochemical parameters, COD and IR images. As we tried it with three different microbial cultures and found them suitable for the purpose, each of culture have their own efficiency of remediation of waste water. For further optimization and standardization of our process can be done after working on the different parameters of the system. It can be applied at higher scale and their results will be appreciable.

**Keywords:** COD, *Pleurotus ostreatus*, *Pleurotus florida*, *Agaricus bisporus*, waste water,

## **Introduction**

India is putting global economic components and pharmaceutical sector. The role of pharmaceutical industry in India is very good for so many years. The Indian pharmaceutical industries are performing very well since one or two decades.

Along with these economic progress in world health care compounds the risk of pollution is also increasing and pharmaceutical industry is not an exception. It became an earnest duty of any human to overcome these endanger and the part of the health environment set up the treatability of pharmaceutical industry waste needs stringent attention.

COD is an established parameter of pollution. So we have tried to bring down COD of pharmaceutical waste water by help of fungal culture. We can easily detect or quantify the amount of organics present in waste water with the help of COD test. The main application of COD is the amount of oxidized pollutants found in waste water.

We are using three different cultures of Fungi as *Pleurotus ostreatus*, *Pleurotus florida* and *Agaricus bisporus*. As these species of fungi are widely available, non-pathogenic, non-toxic, eco-friendly and having such enzymes which are efficient to bring down the COD of waste water State of Gujarat has ascending graph of pharmaceutical product contributing sizable economy to the country. The waste generated by pharmaceutical industry includes numbers of organic compounds which contribute to high COD and renders such waste hazardous. Purpose of the study is to observe the COD and decide the treatment. To select the organism for biological treatment to bring down the COD.

## **Method and Materials**

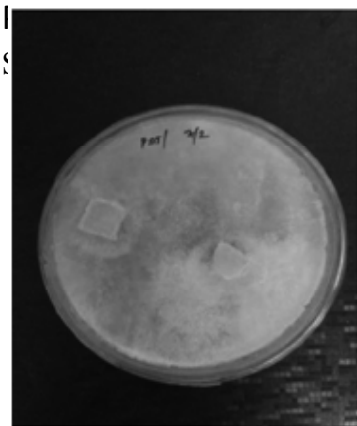
Collection of Sample:

1. Volume - Approx. 5 liter
2. Place – SEZ Pharmaceuticals,  
Changodar,  
Bawla, Ahmedabad.
2. Level –CETP Treatment Plant

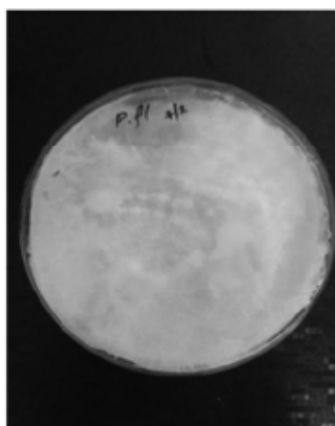
### **Growth of fungi:**

Readily available 3 fungal fruiting body growth as *Pleurotus ostreatus*, *Pleurotus florida* and *Agaricus bisporus* collected from our research laboratory, after that transferred on PDA plate. Placed for incubation at room temperature for 7 days.

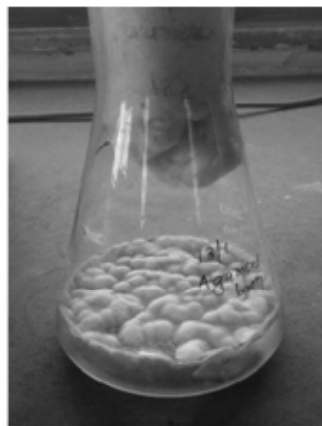
After 7 days observed growth of mycelia of fungi on plates. That used for further worked.



Picture 1:  
*Pleurotus ostreatus*



Picture 2:  
*Pleurotus florida*



Picture 3:  
*Agaricus bisporus*

Dissolved 12.269 g in 1000 ml distilled water.

#### Sulfuric acid reagent:

Add H<sub>2</sub>SO<sub>4</sub> at the rate of 5.5 g Ag<sub>2</sub>SO<sub>4</sub>/kg H<sub>2</sub>SO<sub>4</sub> or 10.12 g silver sulphate/L H<sub>2</sub>SO<sub>4</sub>. Let stand 1 to 2 d to dissolve and mix. This accelerates the oxidation of straight- chain aliphatic and aromatic compounds.

#### Ferroun Indicator Solution:

This indicator is used to indicate change in oxidation-reduction potential of the solution and indicates the condition when all dichromate has been reduced by ferrous ion. It gives a very sharp brown color change which can be seen in spite of blue color generated by the Cr<sup>3+</sup> ions formed on reduction of the dichromate.

#### Standard Ferrous Ammonium Sulfate Titrant (FAS) (0.1 M):

Dissolve 39.2 g Fe (NH<sub>4</sub>)<sub>2</sub>(SO<sub>4</sub>)<sub>2</sub>.6H<sub>2</sub>O in distilled water. Add 20 ml conc. H<sub>2</sub>SO<sub>4</sub>, cool, and dilute to 1000 ml. Standardize solution daily against standard K<sub>2</sub>Cr<sub>2</sub>O<sub>7</sub> digestion solution as follows: Pipet 5.00 ml digestion solution into a small beaker. Add 10 ml reagent water to substitute for sample. Cool to room temperature. Add 1 to 2 drops diluted Ferroun indicator and titrate with FAS titrant.

Mercury Sulphate (HgSO<sub>4</sub>): Crystals or powder

#### Preparation of System:

Take 50 ml of waste water sample in 250 ml of flask, inoculate 8×8 mm growth piece of fungi in aseptic condition in flask then add 0.5% Sugar (Dextrose) in waste water sample.

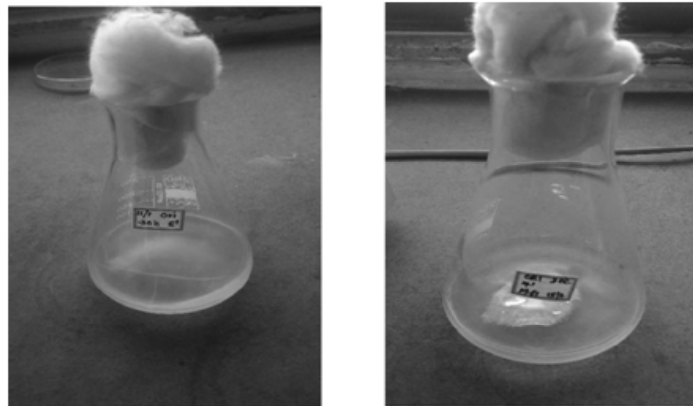
Placed Flask for incubation at 30 p C or 50 p C for 5 or 7 days.



Prepared Systemb for COD reduction

**Observation:**

After incubation of 5 or 7 days observed mycelia growth in the flask.



Picture 5 : After Incubation flasks (Fungal Growth)

**Filtration:**

After incubation filtration of mycelia growth from system by filter paper.

**Dilution:**

Due to presence of biomass of fungi, it gives COD beyond the limit of measurement. So it need to be diluted and then measurement of COD carried out.

**COD measurement:**

There are two methods available for COD determination namely open reflux and closed reflux.

**Open Reflux Principle:**

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Suitable for a wide range of wastes with a large sample size.

Due to its higher oxidizing ability dichromate reflux method is preferred over other procedures using other oxidants.

Oxidation of most organic compounds is up to 95-100% of the theoretical value.

Used open reflux method for COD determination

#### Procedure:

-10 ml  $K_2Cr_2O_7$  + 30 ml Conc.  $H_2SO_4$  + 20 ml Sample + 0.5 g  $HgSO_4$  powder.

(For blank tube add 20 ml distilled water)

-Place it in COD Digester to connect the tubes to condensers and reflux for 2 hours at 150 p C.

-Cool and wash down the condensers with 60 ml distilled water.

-Cool and Titrate against Standard Ferrous Ammonium Sulfate, using as a Ferroin indicator.

-Near the end of titration color change sharply from green blue to wine red Color.

-Note down reading and place it in formula, for calculation of COD

#### Calculation

$$COD, \text{ mg/L} = \frac{(v_1 - v_2) N * 8000}{V_0}$$

Where,  $V_1$  = Volume of  $Fe (NH_4)_2 (SO_4)_2$  required for titration against the blank in mL,

$V_2$  = Volume of  $Fe (NH_4)_2 (SO_4)_2$  required for titration against the Sample in mL,

$N$  = Normality of  $Fe (NH_4)_2 (SO_4)_2$  and

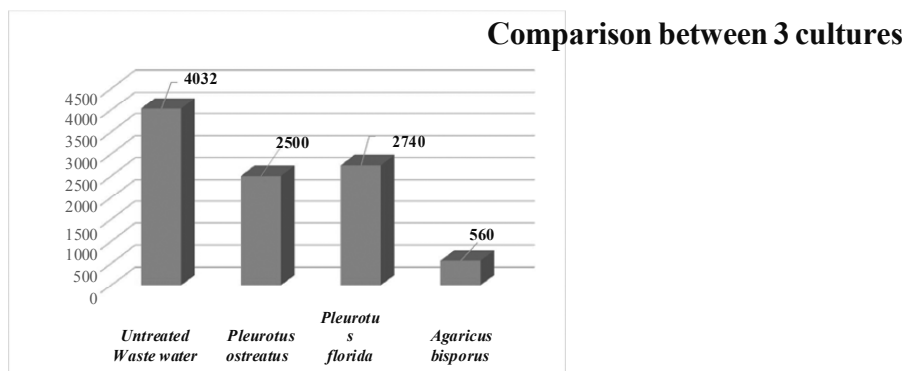
$V_0$  = Volume of Sample taken for testing, in mL

Given table represent results of waste water after treatment, we have used two parameters "with autoclave" and "without autoclave". Initial COD was 4032 mg/L and after treatment with three different cultures we able to bring it down under permissible limit. Results of without autoclave are better than with autoclave. *Agaricus bisporus* given best results while treatment of waste water apart from other two cultures.

**Results & iscusson**

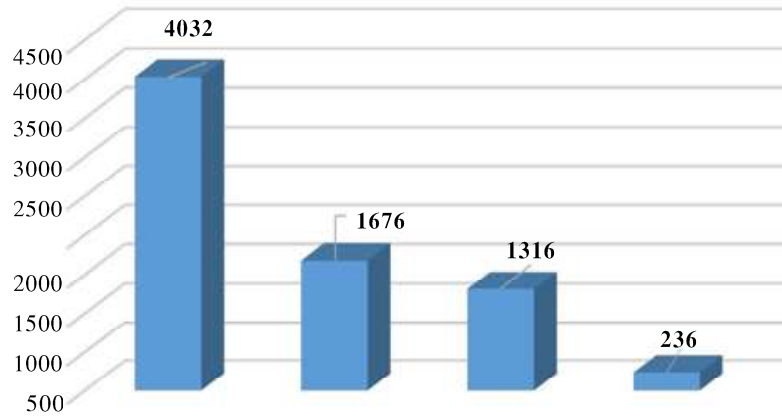
Parameter	Initial COD 4032 mg/L		COD mg/L	
			5th Day	7th Day
Without autoclave	<i>Pleurotus ostreatus</i>	Original	825	214
		Dilution (1:2)	1140	1220
		Original	980	334
With autoclave	<i>Pleurotus ostreatus</i>	Dilution (1:2)	1300	420
		Original	2500	1676
		Dilution (1:2)	2260	1784
	<i>Pleurotus florida</i>	Original	2740	1316
		Dilution (1:2)	2340	1568
		Original	560	198
	<i>Agaricus bisporus</i>	Dilution (1:2)	236	236

Graphical representation of COD reduction:

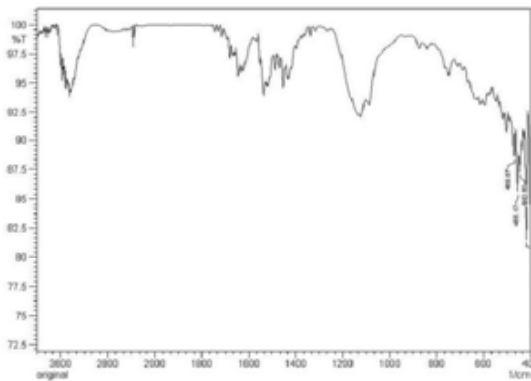


### 5 Days Treatment Results

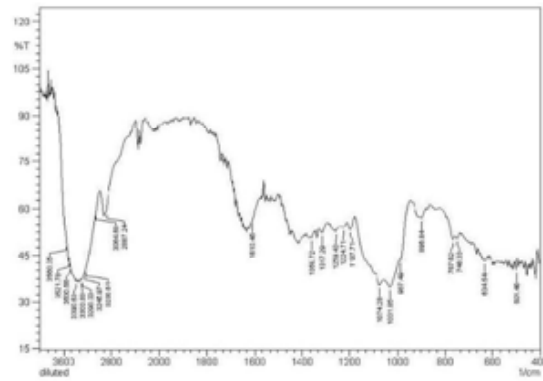
#### Comparison between 3 cultures



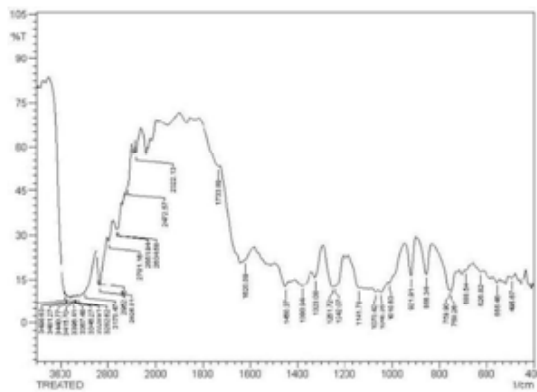
IR images of waste water and after treatment of sample with 3 different culture.



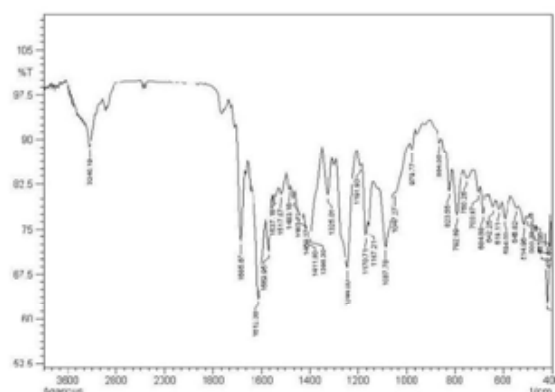
Waste water



Treated - *Pleurotus ostreatus*



Treated- *Pleurotus florida*



Treated - *Agaricus bisporus*

## Conclusion

After work it can be concluding that biological system is efficient for COD reduction of pharmaceutical waste water. Very interesting fungal strains, *Pleurotus ostreatus*, *Pleurotus florida* and *Agaricus bisporus* were selected for its capability to be active in bioremediation process, acting towards several parameters as physico-chemical character, COD, infrared spectroscopy. After analyzing results of all 3 species *Agaricus bisporus* is more efficient than other two species.

## Acknowledgement

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