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## Decolourization and Biodegradation of Distillery Spent Wash by Using Fungi

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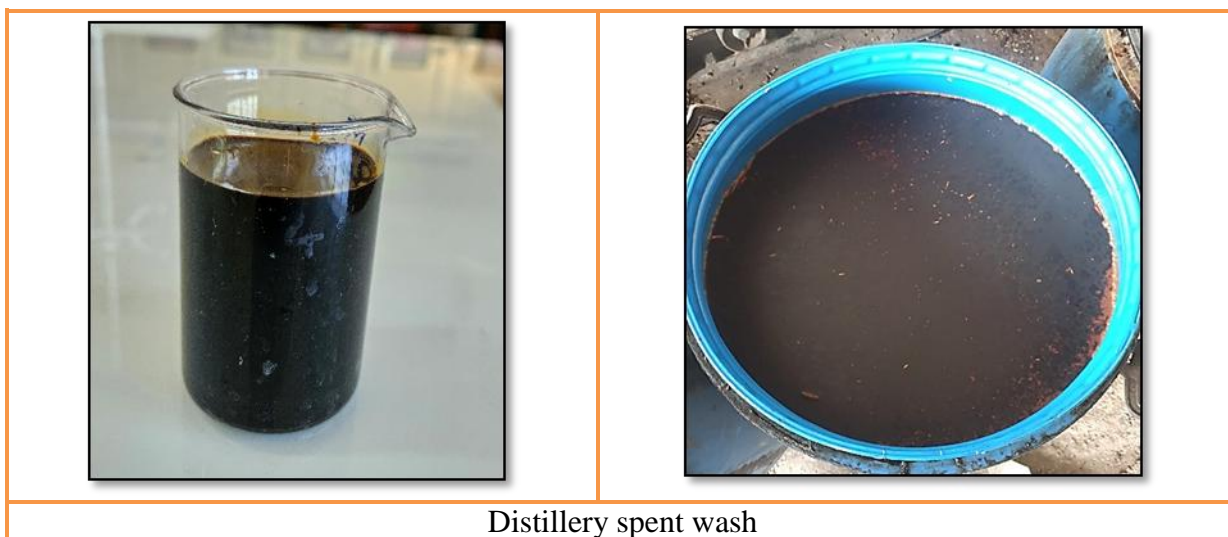
Distillery spent wash is one of the most hazardous industrial effluents generated during alcohol production due to its high organic load, dark brown colour and toxic nature. The presence of melanoidin pigments, high Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) and dissolved solids creates serious environmental problems including soil degradation, water pollution and oxygen depletion in aquatic ecosystems. Conventional physical and chemical treatment methods are often costly and generate secondary pollutants, making them less sustainable for long-term wastewater management. In recent years, fungal biodegradation has emerged as an eco-friendly and efficient alternative for the treatment of distillery spent wash. Fungi possess strong extracellular enzymatic systems capable of degrading complex recalcitrant compounds such as melanoidins and phenolics. Various fungal species including *Aspergillus niger*, *Aspergillus fumigatus*, *Coriolus versicolor* and *Fusarium solani* have shown significant potential in reducing colour, COD and BOD of spent wash. The efficiency of fungal biodegradation is greatly influenced by environmental factors such as pH, temperature, incubation period, spent wash concentration and supplementation of suitable carbon and nitrogen sources.

**Key words:** Decolourization, biodegradation, distillery spent wash, fungi

### Introduction

Distilleries, the alcohol producing industries are one of the major polluting industries as about 88% of its raw material ends up as waste. A typical sugarcane molasses-based distillery generates 15 L of wastewater per liter ethanol produced. This wastewater is known as Distillery Spent Wash (DSW). It is a strong acidic, dark brown coloured hydrophilic viscous liquid waste with strong objectionable odour. Distillery spent wash leads to an environmental pollution due to its high BOD, COD, TSS, TDS, low pH and heavy metals. The dark colour of DSW is due to melanoidins a colour compound generally produced by millard reaction and it reduces light penetration, oxygen depletion in aquatic waterbodies. When discharged on ground it causes serious problems such as soil degradation, groundwater contamination, fertility losses in agricultural land. Conventional physical and chemical treatment methods are often high operation cost and generates secondary pollutants such as sludge. Due to these, biological treatment gained importance as eco-friendly and cost-effective alternative for wastewater remediation. Compared to bacteria, filamentous fungus are less sensitive to changes in temperature, pH, nutrients and oxygen. Fungi are considered highly effective in biodegradation because they produce extracellular enzymes like laccase and peroxidases that degrade melanoidin and other complex organic compounds. Additionally, supplementation of

suitable carbon and nitrogen sources can enhance fungal growth and improve biodegradation efficiency.



Distillery spent wash

### Physico-chemical characteristics of distillery spent wash

Sr. No.	Parameters	Content (mg/L)
1	pH	3.9 - 4.3
2	EC (dS/m)	30.5 - 45.2
3	Biological Oxygen demand	46,100 - 96,000
4	Chemical oxygen demand	1,04,000 - 1,34,400
5	Total dissolved solids	79,000 - 87,990
6	Nitrogen	1,660 - 4,200
7	Phosphorus	225 - 3,038
8	Potassium	9,600 - 17,475
9	Calcium	2,050 - 7,000
10	Magnesium	1,715 - 2,100
11	Sodium	492 - 670
12	Sulphate	3,240 - 3,425
13	Chloride	7,238 - 42,096
14	Zinc	3.5 - 10.4
15	Copper	0.4 - 2.1
16	Manganese	4.6 - 5.1

(Source: Mahimairaja and Bolan, 2004)

### Environmental impact

- **Soil degradation:** increase soil salinity, acidity and organic load, which deteriorates soil structure and fertility.
- **Groundwater contamination:** percolation through soil can contaminate with salts, heavy metal and toxic organic compounds.
- **Water pollution:** discharges in river, lakes or ponds cause pollution due to high BOD, COD, nutrients and dark colour.
- **Oxygen depletion:** high organic matter in DSW reduces dissolved oxygen of aquatic bodies during microbial decomposition.
- **Plant and microbes:** reduces seed germination, inhibit plant growth & microbial population and cause disease in plants.
- **Human and animal health:** consumption of contaminated water or exposed to DSW may cause skin and respiratory disease.

## Conventional treatment methods

### Preliminary Treatment

It is a physical separation of big sized impurities like large solids, debris, plastics, wood, papers *etc.* Main purpose of treatment is to protect pipe, pumps and other equipment from large object in later stage. It involves **1) Screening** : A screen with openings of uniform size is used to remove large solids at the intake point. **2) Grit removal** : Effluent flows through a grit chamber where heavier sand, gravel and small stones settle down. **3) Oil and grease removal** : Skimming tanks separate oils and greases that float to the surface.

### Primary Treatment

It is removal of floating and settleable materials such as suspended solids. Also adjust the pH to make wastewater pH neutral. **1) pH neutralization**: For acidic wastes (low pH): NaOH, Na<sub>2</sub>CO<sub>3</sub>, CaCO<sub>3</sub> and for alkali wastes (high pH): H<sub>2</sub>SO<sub>4</sub>, HCl were used. **2) Sedimentation** : It is the process that bringing together fine particles to form larger flocs, which settle down more quickly. Chemicals like lime, alum (Al<sub>2</sub>(SO<sub>4</sub>)<sub>3</sub>) enhances settling efficiency and makes faster particle removal *via*, sedimentation or filtration.

### Advanced Treatment

It is final cleaning process that improves wastewater quality before it is reused or discharged to the environment. It removes remaining inorganic compounds, heavy metals and pathogens which are harmful to public health. It involves **1) Disinfection** : Chemical (chlorination) or physical (UV) methods to remove pathogens. **2) Filtration** : Sand filter and activated carbon filters. **3) Advance technologies** : Processes like Ultrafiltration, Nanofiltration and Reverse osmosis remove dissolved salts, micro-pollutants and very fine solids.

#### ❖ Limitations of conventional treatments

- ✓ High operational cost
- ✓ Uneconomical for small scale industries
- ✓ Secondary pollutant generation *i.e.* sludge
- ✓ Sometimes incomplete degradation of melanoidin

## Biological treatments

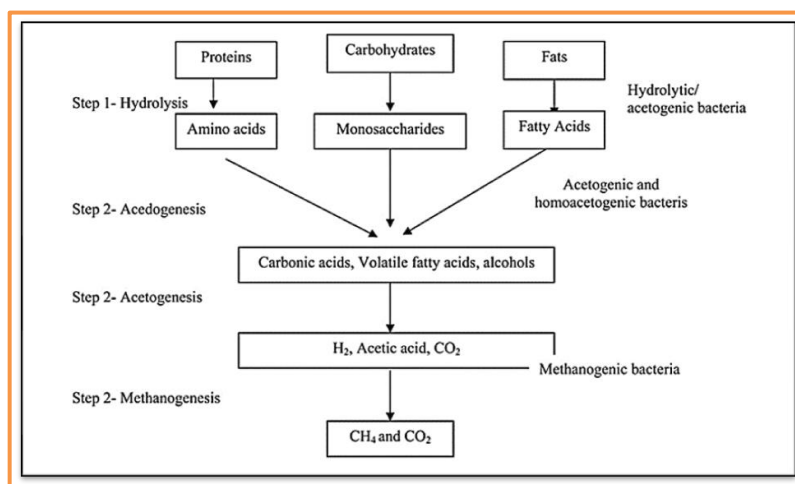
In biological treatment microbes such as bacteria, fungi, yeast, algae were used for degradation. It is relatively inexpensive, environmentally sound and cheap alternative of conventional treatment.

There are two types of biological treatments: i) Anaerobic and ii) Aerobic treatment.

### 1) Anaerobic treatment:

Anaerobic treatment is carried out in the absence of oxygen using anaerobic microorganisms.

During this process, organic matter is converted into methane (CH<sub>4</sub>), carbon dioxide (CO<sub>2</sub>) and stabilized sludge. Therefore, anaerobic digestion have two advantages *i.e.*, it can be used as pollution reduction system and energy generation tool. In anaerobic treatment, microorganisms degrade organic compounds into simpler substances through hydrolysis, acidogenesis, acetogenesis and methanogenesis. This



**Fig: Diagram of anaerobic degradation of distillery spent wash**

process mainly consist of three groups of microorganisms, namely hydrolytic fermentative bacteria, acetogens (acetic acid - forming) and methanogens (methane-forming archaea).

### ❖ **Advantages of Anaerobic Treatment**

- Suitable for high-strength wastewater
- High COD and BOD reduction
- Low sludge production
- Biogas generation
- Lower energy requirement

### ❖ **Limitations of Anaerobic Treatment**

- Incomplete colour removal
- Slow microbial growth
- Sensitivity to pH and toxic compounds
- Post-treatment usually required

## 2) **Aerobic treatment**

Aerobic processes is a biological processes and can be takes place in the presence of oxygen. In this process fungi, bacteria *etc.* widely used. It can decrease the concentration of mineral nutrients through utilization for growth, biomass formation and enzymatic degradation of pollutant. It has been mainly attempted for the decolourization of the major colorant, melanoidins and for reduction of the COD and BOD. Aerobic microorganisms oxidize organic pollutants into CO<sub>2</sub>, water and microbial biomass in the presence of oxygen. Aeration enhances microbial activity and accelerates degradation of pollutants. Here, Fungi are generally superior to bacteria in distillery spent wash treatment because they possess strong extracellular enzymatic systems, higher tolerance to toxic pollutants and better ability to degrade recalcitrant melanoidin pigments. They achieve greater decolourization, COD reduction and detoxification than bacterial systems.

### ❖ **Advantages of Aerobic Treatment**

- Better colour removal
- High degradation efficiency
- Faster microbial growth
- Reduced odor

### ❖ **Limitations of Aerobic Treatment**

- High energy requirement for aeration
- Large sludge production
- Less suitable for highly concentrated wastewater

### **Factors affecting fungal biodegradation:**

- **pH:** pH strongly influences fungal growth, enzyme activity and pollutant degradation. Most fungi involved in spent wash degradation perform efficiently under acidic to neutral condition. *Aspergillus niger* results maximum degradation at 4.0 pH (Sharma *et al.*, 2013). Similarly, *Aspergillus niger* gave maximum decolourization at 6.0 pH (Adikane and Petale, 2014; Wagh and Nemade, 2018; Kale and Shinde, 2020).
- **Temperature:** Temperature affects microbial activity, enzyme secretion and fungal metabolism. Temperature between 30 to 35°C is most favourable for biodegradation. Higher temperature may denature enzymes and inhibit fungal growth, while lower temperatures reduce metabolic activity. *Pseudomonas* sp. Performs best between 30-35°C (Chavan *et al.*, 2006). While, Gupta *et al.* (2014) found that *Fusarium moniliforme* exhibited best decolourization at 30°C. Similarly, kale and Shinde (2020) reported maximum decolourization by *Aspergillus niger* F-2 at 35°C.
- **Incubation period:** Incubation time is important because fungal degradation increases gradually with microbial growth and enzyme production. Insufficient incubation may result in incomplete degradation, while excessively long incubation may reduce efficiency due to nutrient depletion. Kumar *et al.* (2008) reported highest decolourization (84.3%) by *Aspergillus fumigatus* after 15 days of incubation. Pawar *et al.* (2011) reported that *Aureobasidium pullulans* showed maximum degradation at 15 days of incubation. Adikane and Petale (2014) reported maximum degradation by *Aspergillus nidulans* var. *nidulans* at 15 days. Chopra *et al.* (2004) observed maximum

decolourization by *Coriolus versicolor* on the 8<sup>th</sup> day. Moreover, Rane et al. (2018) found that *Fusarium solani* showed maximum degradation after 21 days.

- **Spent wash concentration:** High concentrations of distillery spent wash contain toxic compounds and excessive organic load that inhibit fungal growth and enzyme activity. Therefore, dilution is usually necessary before treatment. Chavan et al. (2013) observed maximum decolourization by *Aspergillus oryzae* in 15% diluted spent wash. Adikane and Patale (2014) reported best COD and colour reduction at 12.5% spent wash concentration using *Aspergillus nidulans*. Rane et al. (2018) found that *Fusarium solani* gave highest degradation at 10% concentration. Thus, optimum dilution of spent wash is necessary to minimize toxicity and enhance fungal biodegradation efficiency.
- **Nutrient supplements:** Fungi require adequate nutrients for growth, reproduction and metabolic activities. Although spent wash contains high organic load, much of the organic matter is present in complex and recalcitrant forms such as melanoidin, which are not easily utilized by fungi. Therefore, external supplementation of carbon and nitrogen sources becomes necessary. Carbon sources provide energy for fungal metabolism, while nitrogen sources support protein synthesis and enzyme production. Among different nutrients, glucose and sucrose are commonly reported as effective carbon sources (0.5-3%), whereas peptone and yeast extract are the most efficient nitrogen sources (0.1-1%). Supplementation significantly improves fungal growth, decolourization efficiency and reduction of pollutants such as BOD and COD. Adikane and Petale (2014) found that sucrose (2%), sodium nitrate (0.5%) gave best results with *Aspergillus nidulans*. While, kale and Shinde (2020) observed that *Aspergillus niger* gave maximum degradation at 2.5% sucrose and 0.1% peptone as carbon and nitrogen source, respectively.

## Conclusion

Fungal biodegradation has proven to be an effective, economical and environmentally sustainable approach for the treatment of distillery spent wash. Compared to conventional treatment methods and bacterial systems, fungi exhibit superior ability to degrade complex organic pollutants and melanoidin pigments through powerful extracellular enzymes. Different fungal strains have demonstrated remarkable efficiency in reducing colour, BOD, COD and toxic compounds under optimized environmental and nutritional conditions. Factors such as pH, temperature, incubation period, spent wash concentration and nutrient supplementation significantly influence the biodegradation performance of fungi. Supplementation with suitable carbon and nitrogen sources enhances fungal growth, enzyme production and overall pollutant removal efficiency. Among the various fungal isolates studied, species of *Aspergillus*, *Fusarium* and *Coriolus versicolor* have shown promising potential for large-scale application in distillery wastewater treatment. Therefore, fungal bioremediation can be considered a promising green technology for minimizing environmental pollution caused by distillery industries and improving sustainable wastewater management practices.

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