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## Role of Microorganisms in Degradation and Detoxification of Distillery Spent Wash

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Sugarcane distilleries convert nearly 90% of raw molasses into spent wash, a hazardous, dark-brown effluent. For every litre of ethanol, 15 litres of this acidic, foul-smelling waste threaten soil and water ecosystems. This environmental crisis stems from recalcitrant melanoidin pigments and extreme organic loads. However, microbial bioremediation offers a sustainable solution. Specialized bacteria, fungi and algae degrade complex pollutants, reducing COD and BOD levels up to 90%. By optimizing pH and temperature, these microorganisms transform toxic sludge into renewable biogas and bio-compost, effectively turning a major industrial pollutant into a valuable, eco-friendly resource.

**Keywords:** Spent wash, degradation, microorganisms, detoxification, melanoidin

### Introduction

Distilleries producing alcohol from sugarcane molasses are among the major agro-industrial polluting industries, as nearly 85–90% of the raw material is converted into waste. A typical molasses-based distillery generates about 12–15 litres of spent wash per litre of ethanol produced. Spent wash is a dark brown, acidic, foul-smelling and highly organic liquid effluent, rich in BOD, COD and dissolved solids. Its intense colour is mainly due to melanoidin pigments, formed through the Maillard reaction, which are highly resistant to degradation. Improper disposal of spent wash causes serious pollution of soil and water resources; therefore, microorganisms play a crucial role in its degradation and detoxification, offering an eco-friendly and sustainable solution for environmental protection.

### Manufacturing process (molasses based)

#### 1. Fermentation:

✓ Molasses is the chief raw material used for production of alcohol.

✓ Molasses contains about 50% total sugars, of which 30 to 33% are cane sugar and the rest are reducing sugar.

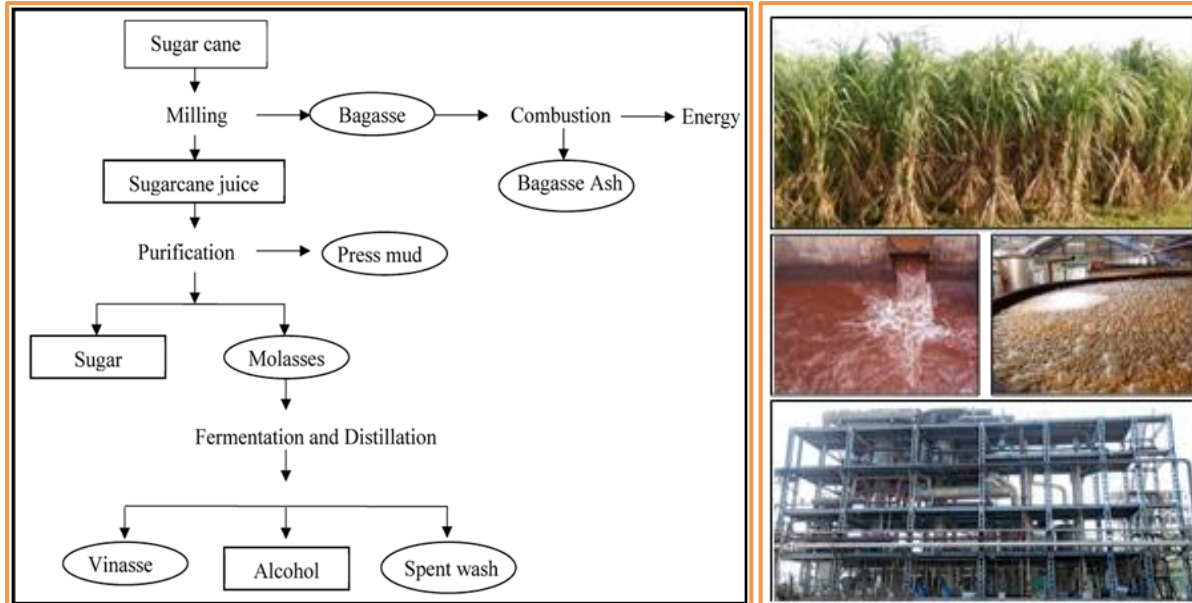
✓ During the fermentation, yeast strains of the species *Saccharomyces cerevisiae*, a living microorganism converts sugar such as sucrose or glucose into alcohol.



## 2. Distillation:

- ✓ After fermentation, the next stage in the manufacturing process is to separate alcohol from fermented wash and to concentrate it to **95%**. This is called Rectified Spirit (RS).
- ✓ For this purpose, method of distillation is employed.
- ✓ After separation of alcohol, the remaining part is the effluent of the process *i.e.* **spent wash and spent lees**.

## Production of Alcohol and Spent wash from sugarcane



## The waste products from sugar mill comprise:

- Bagasse (residue from the sugarcane crushing)
- Pressmud (mud and dirt residue from juice clarification)
- Molasses (final residue from sugar crystallization section)



## Environmental concerns for spent wash

- 1) Water Pollution: Contaminates rivers and lakes, depleting oxygen levels.
- 2) Soil Salinity: Causes soil degradation and reduced fertility.
- 3) Groundwater Contamination: Pollutes drinking water sources.
- 4) Odor Pollution: Produces foul smells during decomposition.
- 5) Air Pollution: Releases methane and other harmful gases.
- 6) Biodiversity Loss: Affects plant and animal life near discharge areas.
- 7) Eutrophication: Leads to algal blooms in water bodies.
- 8) Heavy Metal Contamination: Introduces toxic metals into ecosystems.
- 9) Health Hazards: Causes respiratory and skin diseases in humans.



(a) Spent wash discharged after alcohol production (b) Solid material settle down in the bottom of storage tank as spent wash sludge (c) Large view of the spent wash sludge (d-e) Anaerobically digested spent wash in collection tank (f) Digested sludge discharged after biomethanation of spent wash

### Importance of microorganisms in degradation and detoxification of distillery spent wash

- **Melanoidin Breakdown:** One of the major problems of distillery spent wash is its intense dark brown colour caused by melanoidin pigments. Certain bacteria and fungi have the ability to degrade melanoidin molecules, leading to a significant reduction in colour. Microorganisms such as *Pseudomonas*, *Bacillus* and white-rot fungi produce enzymes that attack these complex pigments, making the effluent less toxic.
- **Heavy Metal Detoxification:** Spent wash contains traces of heavy metals that are harmful to soil, plants and microorganisms. Many microbes can immobilize, absorb or transform toxic metals into less harmful forms through biosorption and bioaccumulation. This microbial activity reduces metal toxicity and prevents their entry into the food chain.
- **Enzymatic Action:** Microorganisms produce a wide range of enzymes such as **oxidases, peroxidases, laccases and dehydrogenases**, which play a key role in breaking down complex organic compounds present in spent wash. These enzymes convert large, resistant molecules into simpler compounds that are easier to degrade, thus accelerating the detoxification process.
- **Organic Matter Degradation:** Distillery spent wash is rich in organic matter, resulting in very high BOD and COD values. Microorganisms utilize these organic compounds as a source of carbon and energy, thereby reducing the BOD and COD levels. It reduce the pollution load of the effluent before its disposal or reuse.
- **Anaerobic Digestion:** Under anaerobic conditions, **methanogenic microorganisms** convert organic matter in spent wash into biogas through the process of methanogenesis. This not only reduces the pollution potential of the effluent but also produces **renewable energy in the form of methane**, making the treatment process economically beneficial.
- **Aerobic Treatment:** In aerobic treatment systems, aerobic bacteria further degrade the remaining organic and hazardous pollutants in spent wash. These microorganisms use oxygen to oxidize organic compounds, resulting in a significant improvement in effluent quality. Aerobic treatment is often used as a polishing step after anaerobic digestion.
- **Phenolic Compound Degradation:** Spent wash contains toxic phenolic compounds that inhibit plant growth and microbial activity. Certain fungi, especially white-rot fungi, are effective in **detoxifying phenolic substances** through enzymatic degradation. This reduces the toxicity of spent wash and enhances its environmental safety.

- **Integrated Microbial Treatment:** The use of certain **microbial consortia**, consisting of different bacteria and fungi, is more effective than single microbial strains. Each microorganism performs a specific function, collectively optimizing colour removal, organic matter degradation and detoxification.

### Microbial mechanisms for degradation of spent wash

Bacterial Mechanisms		
<b>1) Enzymatic Breakdown:</b> <ul style="list-style-type: none"> <li>Bacteria secrete enzymes like amylase, protease and lipase to degrade complex organic pollutants (e.g., proteins, carbohydrates, fats).</li> </ul>	<b>2) COD &amp; BOD Reduction:</b> <ul style="list-style-type: none"> <li>Aerobic bacteria (e.g., <i>Bacillus</i>, <i>Pseudomonas</i>) oxidize organic compounds, lowering COD &amp; BOD.</li> <li>Anaerobic bacteria (e.g., <i>Clostridium</i>) break down pollutants in the absence of oxygen, producing biogas.</li> </ul>	<b>3) Detoxification Efficiency:</b> <ul style="list-style-type: none"> <li>Achieves 70-90% reduction in COD and BOD by metabolizing organic and inorganic pollutants in distillery spent wash.</li> </ul>
Fungal Mechanisms		
<b>1) Complex Organic Degradation:</b> <ul style="list-style-type: none"> <li>Fungi secrete enzymes like cellulase and xylanase to break down cellulose, hemicellulose.</li> </ul>	<b>2) Lignin and Phenolic Breakdown:</b> <ul style="list-style-type: none"> <li>Fungi, particularly white-rot fungi (<i>Phanerochaete chrysosporium</i>), produce lignin peroxidase and manganese peroxidase, breaking down tough organic materials like lignin and toxic phenolic compounds.</li> </ul>	<b>3) Detoxification Efficiency:</b> <ul style="list-style-type: none"> <li>Achieves 60-80% reduction in toxic phenolics, making fungi critical in handling recalcitrant compounds in spent wash.</li> </ul>
Yeast Mechanisms		
<b>1) Organic Acid Assimilation:</b> <ul style="list-style-type: none"> <li>Yeasts (<i>Saccharomyces cerevisiae</i>, <i>Candida tropicalis</i>) metabolize organic acids (acetic acid and lactic acid), reducing effluent acidity.</li> </ul>	<b>2) Fermentation:</b> <ul style="list-style-type: none"> <li>Yeasts convert sugars into ethanol and CO<sub>2</sub>, lowering the organic load in the spent wash.</li> </ul>	<b>3) Detoxification Efficiency:</b> <ul style="list-style-type: none"> <li>Yeasts contribute to 50-70% reduction in organic pollutants.</li> </ul>
Algal Mechanisms		
<b>1) Nutrient Uptake:</b> <ul style="list-style-type: none"> <li>Algae (<i>Chlorella vulgaris</i>) absorb excess N and P from the spent wash and preventing eutrophication.</li> </ul>	<b>2) Oxygenation:</b> <ul style="list-style-type: none"> <li>Algae help to oxygenate the wastewater and improve the overall system health.</li> </ul>	<b>3) Detoxification Efficiency:</b> <ul style="list-style-type: none"> <li>Algae can reduce toxicity by 50%.</li> </ul>


### Factors affecting microbial degradation and detoxification of distillery spent wash

- ✓ **pH level:** Optimal pH is crucial for microbial activity.
- ✓ **Temperature:** Microbial processes depend on suitable temperatures.

- ✓ **Nutrient availability:** Adequate nutrients enhance microbial growth.
- ✓ **Oxygen supply:** Aerobic conditions generally improve degradation.
- ✓ **Microbial diversity:** Diverse communities increase degradation efficiency.
- ✓ **Organic load:** High organic content may inhibit microbial function.
- ✓ **Heavy metals:** Toxic metals can suppress microbial activity.
- ✓ **Retention time:** Sufficient time is needed for effective degradation.
- ✓ **Salinity:** High salt levels can reduce microbial efficiency.

## Uses of Spent wash

<p><b>Irrigation:</b></p> <p>It can be diluted and used for irrigation due to its high nutrient content, though careful management is required to prevent soil salinity.</p>	<p><b>Biogas Production:</b></p> <p>Spent wash can be used in anaerobic digestion to produce biogas, providing a renewable energy source.</p>
<p><b>Composting:</b></p> <p>It serves as an effective additive in compost, accelerating the decomposition process and enriching the compost with nutrients.</p>	<p><b>As a Fertilizer:</b></p> <p>It is rich in nutrients like N, P and K, so it can be applied to fields as a bio-fertilizer, boosting soil fertility and improving crop yields.</p>



## Effect of Bacteria

Chavan *et al.* (2006) found that maximum decolourization (56%) and COD reduction (63%) after 72 hrs was observed in spent wash treated with *pseudomonas sp.* under optimum condition of pH (6.8-7.2) and temperature (30-35 °C). Kaushik and Thakur (2009) observed that maximum reduction in color (21%) and COD (30%) after 12 hrs of inoculation of spent wash with *Bacillus sp.* was obtained in strain 2 (DB2), followed by strain 5 (DB5) and strain 3 (DB3). Chandra *et al.* (2018) revealed that bacterial consortium comprising *Klebsiella pneumoniae*, *Salmonella enterica*, *Enterobacter aerogenes* and *Enterobacter cloacae* showed the optimum decolourization of molasses-melanoidins up to 81% and significant reduction in physico-chemical parameters including heavy metals at optimum temperature (35 °C), pH (8.1) after 168 hrs of incubation. Rane *et al.* (2018) concluded that maximum decolourization (52.61 and 56.75%) and COD reduction (23.65 and 32.51%) of spent wash inoculated with *Bacillus subtilis* in presence of 1.2 % and 1.4% glucose as C and pepton as N source, respectively at 21 day of incubation.

## Effect of Fungus, Yeast and Algae

Chavan *et al.* (2013) studied the decolorization of spent wash by three isolated fungal strains *A.oryzae* , *A. niger* and *Rhizomucor pucillus*; out of which *A. oryzae*, showed maximum (45.7±0.18%) decolorization, BOD reduction (63.9±0.13) and COD reduction (48.7±0.13%) of diluted(15%) spentwash within 96 hrs of incubation. Sharma *et al.*(2013) reported that *Aspergillus niger* achieved 67.7% decolourization and 86.5% COD reduction with glucose supplementation, while without glucose, the rates were lower. While, *Curvularia andropogonis* showed 46.98% decolorization with an added carbon source but had a higher COD reduction (88.35%) without glucose supplementation. Wagh and Nemade (2018) revealed that among the five different cultures (*Aspergillus fumigatus sp.*, *Aspergillus niger gr1* , *Aspergillus niger gr2*, *Aspergillus niger gr3*, *Mixed colonies of Aspergillus oryzae* and *Alternaria sp.*), *Aspergillus niger gr1* showed optimum decolourization (81.5%) and COD reduction(67.89%) of spent wash. Chuppa *et al.* (2020) concluded that among the strains tested in spent wash, the species from the *Aspergillus* and *Trametes* genus gave the best results for bioremediation purposes with COD reductions (76.53% by *Aspergillus terreus* var

*africanus*). Patel et al. (2023) reported that *Candida tropicalis* had the highest capacity of decolourization (83%) among different yeast isolates at 35°C, pH 5.0, within 32 hours of incubation.

### Comparative effect of microorganisms

Singh and Singh (2020) concluded that minimum optical density was observed by C+D consortium on 7<sup>th</sup> day incubation followed by D+E+F, A+B+C+D+E+F, A+B+C, E+F and A+B in which A is *E.coli*, B is *Pseudomonas aeruginosa*, C is *Staphylococcus aureus*, D is *Serratia odoriferae*, E is *Proteus vulgaris* and F is *Candida albicans*. Patil and Pathade (2023) stated that *Klebsiella grimontii* has the potential to stabilize the spent wash to a maximum degree by reduction in COD and BOD with corresponding decrement of 95.31% and 81.39%, respectively.

### Conclusion

Molasses-based distilleries generate highly polluted spent wash rich in BOD, COD, colour, phenolics and toxic substances, which can severely harm soil and water if discharged untreated. Microorganisms such as bacteria (*Pseudomonas*, *Bacillus*), fungi (*Aspergillus*), yeasts (*Candida tropicalis*) and algae (*Chlorella vulgaris*) play a crucial role in its degradation and detoxification by reducing organic load, breaking down melanoidins and phenolic compounds, removing heavy metals and lowering toxicity. Research shows significant reductions in colour, BOD and COD, especially under optimized conditions. Therefore, microbial treatment is an eco-friendly, sustainable and effective approach for the management of distillery spent wash and environmental protection.

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