Abstract

Distilleries are one of the most polluting industries generating large volume of wastewater having a serious environmental concern. Distillery effluent is characterized by dark brown color, acidic pH, high temperature, low dissolved oxygen (DO), high biochemical oxygen demand (BOD) and chemical oxygen demand (COD). Distillery wastewater disposed onto the environment prior to treatment is hazardous and leads to soil and water pollution. The dark brown color of distillery effluent causes reduction of sunlight penetration, decreased photosynthetic activity and dissolved oxygen concentration in rivers, lakes and lagoons, hence becomes detrimental to aquatic life. It also causes reduction in soil alkalinity and inhibition of seed germination. Different physicochemical and biological methods have been investigated for the treatment of distillery effluents. In recent years, increasing attentions has been directed towards biological wastewater treatment methods. Bioremediation of wastewater using microorganisms is efficient and cost effective method. Microorganisms as bacteria, fungi, and algae have been shown to exhibit bioremediation activities mainly due to their production of complex and non-specific enzymatic systems capable of degrading various forms of pollutants from wastewater. The main concern of the present review is also to explore the role of microorganisms in wastewater treatment disposed from distilleries. Further, the mechanisms of color removal by fungi, bacteria and algae have also been incorporated.

Keywords: Microbial; Physicochemical; Treatment; Wastewater

Introduction

Distilleries can be categorized among the most polluting industries generating large volume of wastewater known as spent wash [1]. Distilleries generate wastewater at various stages in the manufacturing process as distillation, condenser cooling, fermenter cooling, fermentation and washing stages. Larger amount of the effluent is produced at distillation and condenser cooling stages [2]. The characteristics of the wastewater generated depend on the feedstock used. Distilleries are agro-based industries, which utilize agricultural products as sugar cane juice, sugar cane molasses, sugar beet molasses, corn, wheat, cassava, rice, barley as raw materials [3,4].

Distillery effluent is characterized by its acidic pH (4-5), dark brown color, high temperature (53-100°C), low dissolved oxygen (DO), high biochemical oxygen demand (BOD) (40,000-50,000 mg/L) and chemical oxygen demand (COD) (80,000-100,000 mg/L) [5]. Apart from the high BOD and COD load, distillery effluent also contains significant amount of phenols (7,202 mg/L), chlorides (7,997 mg/L), sulphates (1,100 mg/L), nitrates, phosphates (1,625 mg/L) and heavy metals [6,7]. The dark brown color of the effluent is mainly due to the formation of polymer melanoidin by a non-enzymatic browning reaction called Maillard reaction [8]. Melanoids are highly recalcitrant and have antioxidant properties which make them toxic to many microorganisms [9].

Distillery wastewater disposed to the environment prior to treatment is hazardous and can be a major source of soil and water pollution. It induces toxic substances into water bodies as rivers, lakes and lagoons which adversely affect aquatic plants and animals. The highly colored nature of the effluent also leads to the reduction of sunlight penetration in rivers, lakes or lagoons which, in turn, reduces oxygenation of the water by photosynthesis and hence becomes detrimental to aquatic life [10]. Disposal of distillery wastewater into soil is equally harmful, as it reduces soil alkalinity and manganese availability [11]. It also imparts high concentration of heavy metals viz., copper, nickel, silver, cadmium, iron and mercury which are capable of inhibiting seed germination and seedling growth [12,13]. According to various reports, application of distillery effluent for irrigation without proper monitoring might result in reduction of soil fertility by suppressing the activity of soil microorganisms as nitrogen fixing bacteria rhizobium and azotobacter [14,15].

Distillery effluent must be treated before it is disposed into the environment which helps to minimize the adverse effect posed by the effluent. There have been several treatment technologies explored so far for the reduction of the pollutants from distillery wastewater. Treatment methods can vary based on the chemical composition of the effluent as well as economic viability of the technology. Generally, wastewater treatment methods can be categorized as physical, chemical and biological methods. Physicochemical treatment methods such as adsorption, sedimentation, screening, coagulation, pH adjustment, reverse osmosis, ultrafiltration, flotation, oxidation, electrolysis, membrane filtration and evaporation have been used for treatment of distillery effluents.

Physicochemical methods of wastewater treatment have so many drawbacks such as consumption of chemicals, high cost, large amount of sludge left after treatment, and possible formation of harmful by-products [16]. As a result of this, in recent years, biological wastewater treatment using microorganisms has attracted the attention of researchers all over the world. Microbial degradation and decolorization of distillery effluents have been found as cost effective and environmentally friendly alternative to physicochemical methods. Various types of microorganisms as bacteria, fungi, and algae have been reported for their potential in degradation and decolorization of various
industrial effluents including that of distilleries. Hence, this review discusses and summarizes the role of microorganisms in degradation and decolorization of distillery wastewater. Moreover, the mechanisms of microbial degradation of melanoidin by fungal, bacterial and algal systems are also discussed.

**The Role of Microorganisms in Effluent Treatment**

Microorganisms play a key role in bioremediation process and have been proven as an efficient, low cost and environmental friendly alternative to physicochemical methods. Several microbial species including fungi, bacteria and algae have been studied for their capacity to degrade and decolorize toxic chemical pollutants present in various industrial wastewater including distilleries. Free or immobilized cells have been studied widely for bioremediation of distillery wastewater. Immobilizing microorganism on inert support material including alginate, polyacrylamide, agar, polystyrene, and polyurethane is more advantageous compared to that of free-cell. Some of the advantages include compact physical structure of carrier pellets, high biomass retention, reusability of culture and easier separation process [17,18]. The potential of microorganisms in distillery wastewater treatment is highly dependent on the type of chemical composition of wastewater, nutrient, pH, temperature, oxygen and inoculum size [19,20].

**Bacterial treatment**

A wide variety of bacterial cultures as *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Lactobacillus plantarum*, *Bacillus circulans*, *Bacillus megaterium*, *Bacillus firmus*, *Bacillus thuringiensis*, *Bacillus cereus*, *Lactobacillus hilgardii*, *Lactobacillus cornyiformis*, *Xanthomonas fragariae* have been reported for their activity in degradation and decolorization of pollutants from distillery effluents. Table 1 presents some of the bacterial cultures involved in biodegradation of distillery wastewater.

| Wastewater Composition | Microorganism | Treatment Process | Initial COD Load (g/L) | COD Removal Efficiency (%) | Treatment Time (days) | Ref |
|------------------------|---------------|------------------|------------------------|---------------------------|----------------------|-----|
| Color (dark brown), pH (2-4), COD (146.38 g/L), TDS (8.9 g/L), sulphate (1.1 g/L), phosphorus (5.1 g/L), free chlorine (5.84 g/L) | *Pseudomonas* sp. | A pure culture of the isolate was transferred in medium with 10% diluted spent wash and incubated at room temperature. | 146.4 | 63 | 3 | [6] |
| pH (7.5), COD (12.1 g/L), BOD (6.88 g/L), TS (17.8 g/L), nitrogen (0.98 g/L), phosphorus (0.38 g/L) | B. cereus | Each bacterial isolate was immobilized on sodium alginate and degradation of anaerobiologically digested distillery wastewater was carried in batch experiment. | 30 | 81 | 2 | [1] |
| pH (7.5-8), BOD (40 g/L), COD (100 g/L), TDS (38.4 g/L), TSS (105 g/L), TVA (2.8 g/L) | B. megaterium | Bioremediation of molasses spent wash was carried in medium supplemented with glucose, yeast extract, KH2PO4 and MgSO4.7H2O and kept for incubation for 15 days. | 100 | 80.8 | 15 | [22] |
| BOD (52.5 g/L), COD (112 g/L), TS (2.1 g/L), TDS (1.985 g/L), TSS (0.07 g/L), sulphate (6.26 g/L), phosphate (0.0024 g/L), phenol (0.584 g/L) | Mixed culture of: B. thuringiensis, B. brevis, Bacillus sp. (MTCC6506) | The decolorization of Sucrose-Glutamate-Acid (SGA) was carried out by the mixed bacterial culture supplemented with 15% glucose under shaking flask conditions (150 rpm) at pH 7.0 and 37°C. | 112 | 63.39 | 1 | [47] |
| pH (7.5), COD (20.6 g/L), TS (29.6 g/L), TN (1.7 g/L), Total phosphorus (0.1 g/L) | *Lactobacillus* L-2 | The isolated bacteria was supplemented with 10 g/L glucose and bio-remediate 12.5% diluted anaerobiologically digested spent wash. | 20.6 | 57 | 7 | [10] |
| pH (7.5-8), BOD (8-10 g/L), COD (45-52 g/L), TS (72.5 g/L), TSS (40.7 g/L), phosphates (1.625 g/L), sulphates (3.875 g/L), chlorides (8 g/L), phenols (7.2 g/L) | A bacterial consortium of: *P. aeruginosa* A01, *S. maltophilia*, *P. microbiris* | The degrading and decolorizing of anaerobiologically treated distillery wastewater was studied using isolated bacterial consortium. | 45 | 51 | 3 | [7] |
| pH (8.2), BOD (3 g/L), COD (54 g/L), phosphorus as P (0.07 g/L), TS (45 g/L) | Acetogenic bacteria of strain No. BP103 | Decolorization of molasses wastewater was carried in replacement culture system. | 54 | 70.9 | 7 | [65] |

**Note:** Total Solid (TS); Biological Oxygen Demand (BOD); Chemical Oxygen Demand (COD); Total Volatile Acid (TVA); Total Suspended Solid (TSS); Total Nitrogen (TN)

Table 1: Bacterial Cultures Employed for Treatment of Distillery Effluent.
optimum condition. Another facultative anaerobic bacterial culture L-2 belonging to Lactobacillus showed similar decolorization of 31% for 12.5% (v/v) diluted digested spent wash in 7 days of incubation. Along with decolorization this bacterial culture also removed 56.2% COD [10]. Nakajima et al. [29] observed decolorization yield of 35.5% using bacterial strain MD-32 within 20 days of cultivation under both thermophlic and anaerobic conditions. The COD and color removal efficiency of anaerobic bacterial strains is lower than that of aerobic bacteria. Hence, it is important to isolate bacterial strains capable of degrading and decolorizing toxic chemical pollutants under anaerobic conditions.

### Fungal treatment

In recent years, several fungal cultures have been investigated for their ability to degrade and decolorize distillery wastewater. Table 2 presents some of the fungal cultures involved in bioremediation of distillery wastewater. One of the most studied fungi having high molasses wastewater bioremediation activity belongs to the genera of Aspergillus. Miranda et al. [30] studied color elimination from anaerobically-aerobically treated beet molasses spent wash using Aspergillus niger. The fungal culture showed COD and color removal yield of about 65 and 75%, respectively when supplemented with 10 g/L sucrose, 1.8 g/L NH₄NO₃, 1 g/L KH₂PO₄, and 0.5 g/L MgSO₄·7H₂O with an initial pH of 5. In the culture with the optimal nutrient concentration 83% of the total color removed was eliminated biologically and 17% by adsorption on the mycelium. Ohomomo et al. [31] used mycelia of a thermophilic strain Aspergillus fumigatus G-2-6 for batch and continuous decolorization of melanoidin solution. This strain decolorized about 75 and 70% of a molasses melanoidin solution under batch and continuous culture, respectively when the strain was cultivated on a glycerol-peptone medium at 45°C within 3 days. At the same time, about 51% of the chemical oxygen demand and 56% of the total organic carbon in the initial solution were removed. Later on they observed similar decolorization yield of 75% using autoclaved mycelium of Aspergillus oryzae No. Y-2-32 when it was cultivated at 35°C for 4 days on glycerol-peptone medium with shaking. The main melanoidin decolorization activity of this strain was due to the adsorption of melanoidin to mycelia. This fungal strain adsorbed lower weight fractions of melanoidin and degree of adsorption was influenced by the kind of sugars used for cultivation [28].

White-rot fungi are among most widely exploited microorganisms because of their capacity in bioremediation of toxic compounds. They produce various forms of complex and non-specific intracellular and extracellular enzymatic system including laccases, manganese peroxidases, lignin peroxidase, and sugar oxidase involved in the degradation of various toxic pollutants [32,33]. The most widely studied white-rot fungal species in bioremediation of distillery wastewater are Phanerochaete sp., Flavodon sp., Coriolus sp. and Trametes sp. Among white-rot fungi the highest melanoidin decolorization in a range of 80-82% have been reported for Coriolus sp. No 20 [34,35], Coriolus versicolor Paà [36], Trametes versicolor and Trametes hirsuta [37] under optimum conditions. Along with decolorization and COD removal, white-rot fungi are also effective in removing phenolic compounds from distillery wastewater. It has been reported that Trametes pubescens MB 89 and Phanerochaete chrysosporium can remove 80 and 63% total phenolic compounds from wastewater [38,39]. In another report Flavodon flavus removed benzo(a)pyrene a polycyclic aromatic hydrocarbons (PAHs) 68% from molasses spent wash within 5 days [18].

### Algal treatment

Microalgae are unicellular microorganisms that are known for their capacity in biodesorption and biodegradation of toxic chemical pollutants as phenols, heavy metals, pesticides, polycyclic aromatic hydrocarbons (PAHs), xenobiotics and melanoidins from wastewater [40,41]. Utilizing microalgae for bioremediation purpose is advantageous compared to that of bacterial and fungal systems in many ways. The first advantage is that, microalgae has a great potential in utilizing contaminants as ammonium, nitrate and phosphate as a nutrient hence minimizes the amount of externally added nutrient in case of fungi and bacteria. Secondly fungi and bacteria require optimum condition for growth and bioremediation activity whereas microalgae can grow rapidly and adapt harsh conditions. Thirdly microalgae produces valuable products such as ethanol, methane, livestock feed, or it can also be used as organic fertilizer due to its high N:P ratio [42]. Hence, utilizing microalgae for bioremediation purpose

| Wastewater Composition | Microorganism | treatment Process | Initial COD Load (g/L) | COD Removal Efficiency (%) | Treatment Time (days) | Ref |
|------------------------|--------------|-------------------|------------------------|---------------------------|----------------------|-----|
| Color (greenish dark brown), pH (7.2), BOD (5.65 g/L), COD (34.8 g/L), TDS (4.5-6.62 g/L), sulphates (0.16 g/L), free chlorine (0.8 g/L) | C. cladosporioides | The fungus was used in a batch experiment to decolorize 100 mL of 15 % diluted ADSW cultivation medium supplemented with carbon and nitrogen source. | 34.8 | 62.5 | 10 | [64] |
| pH (5.2), COD (80.5 g/L), TS (109 g/L), TSS (3.6 g/L), sulfates (5.5 g/L), Total phenols (0.45 g/L) | Penicillium sp. P. decumbens | Aerobic degradation of beet molasses alcoholic fermentation wastewater diluted to 50%. | 52.1 | 50.7 | 5 | [61] |
| Color (brown), pH (3.65), BOD (40 g/L), COD (100 g/L), TDS (38.4 g/L), TSS (105.2 g/L), COD (2.9 g/L) | A. fumigatus | Aerobic degradation of beet molasses alcoholic fermentation wastewater diluted to 50%. | 100 | 84.0 | 15 | [22] |
| Color (dark-brown), pH (4.1), COD (0.06 g/L), TS (52.4 g/L), SS (12.8 g/L) | A. terreus | Aerobic degradation of beet molasses alcoholic fermentation wastewater diluted to 50%. | 0.06 | 61.7 | 7 | [32] |
| Color (dark-brown), pH (4.3), BOD (42 g/L), COD (80 g/L) | Trametes sp. I-62 | Aerobic degradation of beet molasses alcoholic fermentation wastewater diluted to 50%. | 25.5 | 79 | 15 | [33] |
| Color (greenish dark brown), pH (7.2), BOD (5.65 g/L), COD (34.8 g/L), TDS (4.5-6.62 g/L), sulphates (0.16 g/L), free chlorine (0.8 g/L) | P. chrysosporium | The fungus was used in a batch experiment to decolorize 100 mL of 15 % diluted ADSW cultivation medium supplemented with carbon and nitrogen source. | 34.8 | 62.5 | 10 | [64] |
| pH (5.2), COD (80.5 g/L), TS (109 g/L), TSS (3.6 g/L), sulfates (5.5 g/L), Total phenols (0.45 g/L) | Penicillium sp. P. decumbens | Aerobic degradation of beet molasses alcoholic fermentation wastewater diluted to 50%. | 52.1 | 50.7 | 5 | [61] |
| Color (brown), pH (3.65), BOD (40 g/L), COD (100 g/L), TDS (38.4 g/L), TSS (105.2 g/L), COD (2.9 g/L) | A. fumigatus | Aerobic degradation of beet molasses alcoholic fermentation wastewater diluted to 50%. | 100 | 84.0 | 15 | [22] |
| Color (dark-brown), pH (4.1), COD (0.06 g/L), TS (52.4 g/L), SS (12.8 g/L) | A. terreus | Aerobic degradation of beet molasses alcoholic fermentation wastewater diluted to 50%. | 0.06 | 61.7 | 7 | [32] |
| Color (dark-brown), pH (4.3), BOD (42 g/L), COD (80 g/L) | Trametes sp. I-62 | Aerobic degradation of beet molasses alcoholic fermentation wastewater diluted to 50%. | 25.5 | 79 | 15 | [33] |

**Note:** Total solid (TS); Biological Oxygen Demand (BOD); Chemical Oxygen Demand (COD); Total Volatile Acid (TVA); Total Suspended Solid (TSS); Anaerobically Digested Spent Wash (ADSW); Suspended Solids; (SS); Not Recorded (NR)

**Table 2:** Fungal Cultures Employed for Treatment of Distillery Effluent.
The mechanism of microbial degradation of melanoidin is difficult to inspect since its chemical structure is yet to be fully discovered. However, several studies revealed the role of microorganisms in degradation and decolorization of melanoidin from distillery wastewater. Different mechanisms of color removal from distillery effluent have been inspected. Melanoidin removal by microorganisms can take place through enzymatic degradation, utilizing the pigment as a carbon and nitrogen source, flocculation by microbially secreted substances, and adsorption onto the surface of living (resting) and dead (autoclaved) cells [6,48,50,51]. Various forms of intracellular and extracellular enzymes as lactases, manganese peroxidases, lignin peroxidase, sugar oxidases such as sorbose oxidase have been reported to show melanoidin degradation activity [37,52].

The melanoidin decolorization mechanism by adsorption onto the surface of both living (resting) and dead (autoclaved) cells have been reported for Rhizoctonia sp. D-90 [53], Aspergillus fumigatus G-2-6 [31], Aspergillus oryzae Y-2-32 [28], Coriolus No.20 [35], Coriolus versicolor Pe4a [36,54], aceticogenic bacteria BP103 [27] and Lactobacillus plantarum No. PV71-1861 [55]. The melanoidin decolorization mechanism involved adsorption onto mycelium first, then incorporated into the cell and then decomposed by intracellular enzyme which require active oxygen molecules and sugar in reaction mixture [35,36,54]. Watanabe et al. [34] purified enzymes from Coriolus sp. No.20 which was identified as sorbose oxidase and involved in melanoidin decolorization activity. It was suggested that melanoidins were decolorized by the active oxygen such as hydrogen peroxide (H₂O₂) species produced by the enzymatic oxidation reaction with sugar oxidase (L-sorbose oxidase and Glucose oxidase) in the presence of sugar such as glucose, maltose, sucrose, lactose, sorbose, galactose and xylose as a substrate [34,56].

Microorganisms particularly white-rot fungi produces various forms of nonspecific extracellular enzyme including H₂O₂, lactase and oxidases namely lignin peroxidases (LiP) and manganese peroxidase (MnP). Lignin peroxidase (LiP) catalyzes the oxidative degradation of lignin by H₂O₂. Both lignin peroxidases (LiP) and manganese peroxidase (MnP) oxidizes a variety of substrates including Mn(II), phenolic and non-phenolic compounds and different types of dyes [57]. LiP and MnP differ in their catalytic mechanism where the former catalyzes one-electron oxidation of phenolic and non-phenolic compounds by H₂O₂, promoting production of the corresponding free radicals while the latter catalyzes H₂O₂ dependent oxidation of Mn(II) to Mn(III) and the oxidized Mn(III) then catalyzes one-electron oxidation of phenolic and non-phenolic compounds by H₂O₂, promoting production of the corresponding free radicals [49,58]. The free radicals generated by the two mechanisms are responsible for the degradation of wide variety of pollutants including melanoidins. Production of H₂O₂, lactase, manganese-dependent peroxidase (MnP) and lignin peroxidase (LiP) have been reported in several fungal, bacterial and algal species as Bacillus licheniformis, Alcaligenes sp., Penicillium pinophilum, Alternaria gaisen, Coriolus hirsutus, Emericella nidulans, Flavodon flavus, Oscillatoria boryana BDU 92181 and Neurospora intermedia [18,46,49,56,59,60].

Conclusion

Microorganisms as fungi, bacteria and algae plays key role in bioremediating toxic pollutants from distillery wastewater for safe disposal. There are many reports showing the activity of microorganism in biodegrading and decolorizing distillery wastewater. However, large number of reports on fungal, bacterial and algal treatment has been limited to laboratory-scale experiments. The application of the process to full-scale was still inconvenient due to lack of stability, nutrient supplement, long growth cycle, spore formation, loss of extracellular...
enzymes and lack of an appropriate reactor system. Thus, application of the process to field scale would need further research. Moreover, it is also found necessary to isolate, characterize and genetically improve microbes for better bioremediation yield [61-65].

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Dedicated to my wife Hirut Mamo and my daughter Obse Terefe.

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