

Review Article

The Role of Microorganisms in Distillery Wastewater Treatment: A Review

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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 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 feed stock 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]. Melanoidins 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 seeding 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 environmental 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

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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 coryniformis, 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.

A wide variety of aerobic or anaerobic bacterial strains have been involved in bioremediation of distillery wastewater. However, a large number of bacterial species including Bacillus sp., Pseudomonas sp., Alcaligenes sp. and acetogenic bacteria operates effectively under aerobic conditions. Tiwari et al. [21] isolated thermotolerant bacterial culture comprised of Bacillus subtilis, Bacillus cereus and Pseudomonas aeruginosa from soil contaminated with distillery wastewater. Among which Bacillus subtilis showed maximum decolorization 85% at 45°C in the presence of little amount of carbon (0.1%, w/v) and nitrogen sources (0.1%, w/v) within a very short incubation period 24 hr. Bacillus cereus and Pseudomonas sp. showed 73 and 69% decolorization, respectively under optimum conditions. Bacillus subtilis showed best thermotolerance ability and could tolerate 35-50°C without affecting exponential growth phase. According to reports from different investigations the genus Bacillus showed the highest bioremediation efficiency compared to other bacterial cultures. Various strains of Bacillus sp. showed an average decolorization 75-81%, COD 80-85% and BOD 85-95% removal efficiency whereas other species as Alcaligenes sp., Pseudomonas sp. and acetogenic bacteria removed color by about 50-78%, COD 62-76% and BOD 70-82% under optimum conditions [1,6,22-27].

Aerobic bacterial strains are very effective in bioremediating distillery effluents under aerobic conditions. However, those bacterial strains are not economical due to high energy consumption for aeration thus; it was very difficult to apply those bacterial strains on an industrial scale. Considering this problem it is important to isolate bacterial strains that can degrade distillery wastewater under anaerobic condition. Anaerobic bacterial strains are advantageous than that of aerobic strains due to low energy consumption hence, minimizes cost of wastewater treatment. Ohmomo et al. [28] reported the first bacterial strain Lactobacillus hilgardii W-NS capable of decolorizing molasses melanoidins under anaerobic condition. This bacterial strain decolorized about 28% of molasses melanoidin under

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 (7.6), 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 anaerobically digested distillery wastewater was carried in batch experiment.	30	81	2	[1]
	X. fragariae			76		
	B. megaterium			76		
Color (brown), pH (3.85), BOD (40 g/L), COD (100 g/L), TDS (38.4 g/L), TSS (105 g/L), TVA (2.8 g/L)	B. circulans	Bioremediation of molasses spent wash was carried in medium supplemented with glucose, yeast extract, KH ₂ PO ₄ and MgSO ₄ .7H ₂ O and kept for incubation for 15 days.	100	80.8	15	[22]
	B. megaterium			80.9		
	B. firmus			78.9		
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 anaerobically 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: <i>P.</i> <i>aerugenosa</i> A01, <i>S. maltophia, P.</i> <i>microbilis</i>	The degrading and decolorizing of anaerobically treated distillery wastewater was studied using isolated bacterial consortium.	45	51	3	[7]
pH (8.2), BOD (3 g/L), COD (54 g/L), phosphorous 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]

Table 1: Bacterial Cultures Employed for Treatment of Distillery Effluent.

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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 thermophilic 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 strains 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 anaerobic-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 KH, PO, and 0.5 g/L MgSO, 7H, O 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 glycerolpeptone 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 Page 3 of 6

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 Ps4a [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 chrysospotium 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 bioadsorption 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 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	reatment Process	Initial COD Load (g/L)	COD Removal Efficiency (%)		Ref
Color (greenish dark brown), pH (7.2), BOD (5-6.5 g/L), COD (34.8 g/L), TDS (4.5-4.62 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 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	57	5	[61]
Color (brown), pH (3.85), BOD (40 g/L), COD (100 g/L), TDS (38.4 g/L), TSS (105.2 g/L), TVA (2.8 g/L)	A. fumigatus	Bioremediation of molasses spent wash was carried in	100	84.0	15	[22]
	A. terreus	medium supplemented with glucose, yeast extract, KH_2PO_4 and $MgSO_4.7H_2O$ and with fungi and kept for incubation for 15 days.		84.0		
Color (dark-brown), pH (4.1), COD (0.06 g/L), TS (52.4 g/L), SS (12.8 g/L)	Trametes sp. I-62	20% (v/v) of distillery effluent was added to the culture medium and incubated for 7 days at 28° C under sterile condition.	0.06	61.7	7	[32]
NR	P. chrysosporium	The fungus was immobilized on different support materials, such as polyurethane foam (PUF) and scouring web (SW), in rotating biological contactor (RBC).	NR	48	17	[39]
Color (dark brown), pH (4.3), BOD (42 g/L), COD (80 g/L)	F. flavus	The isolated fungi was immobilized on polyurethane foam cube and decolorized 10% diluted molasses wastewater.	80	50	5	[18]
Total phenols (0.54 g/L), pH (3.9), COD (25.5 g/L)	T. pubescens MB 89	The isolated fungus was used in flask cultures and a bubble lift bioreactor to treat 10% diluted wastewater.	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.

is advantageous compared to fungal and bacterial system. Various species of microalgae as *Chlorella vulgaris*, *Oscillatoria boryana*, *Chlorella pyrenoidosa*, *Chlorella sorokiniana*, *Coenochloris pyrenoidosa*, *Nostoc muscorum*, *Neochloris oleoabundans*, *Phormidium valderianum*, *Chlorella zofingiensis*, and *Chlorella ellipsoidea* have been used in bioremediation of wastewater.

The green microalgae belonged to the genera of Chlorella have been studied most widely due to its capacity of bioremediating toxic chemicals pollutants. Valderrama et al. [43] carried out research to develop a procedure for treatment of recalcitrant wastewater from ethanol and citric acid production using first the microalga Chlorella vulgaris followed by the macrophyte Lemna minuscula. In the first stage of treatment, Chlorella vulgaris resulted in a reduction of ammonium ion 71.6%, phosphorus 28% and chemical oxygen demand 61% from 10% diluted wastewater within 4 days of treatment. Travieso et al. [44] evaluated the performance of a laboratory-scale microalgae pond for secondary treatment of distillery wastewater previously digested in an anaerobic fixed bed reactor using Chlorella vulgaris SR/2. Chlorella vulgaris SR/2 removed volatile suspended solids (VSS) 78.8%, total solids (TS) 60.6%, total suspended solids (TSS) 53.4%, chemical oxygen demand (COD) 83.2% and biochemical oxygen demand (BOD) 88.0% from the effluent. More recently, Solovchenko et al. [45] investigated phycoremediation of alcohol distillery wastewater with a novel Chlorella sorokiniana strain isolated from White Sea. This algal strain showed maximum reduction in chemical oxygen demand (COD) 92.5%, nitrate 95%, phosphate 77% and sulfate 35% within four days. Another marine cyanobacterium Oscillatoria boryana decolorized pure melanoidin pigment (0.1%) and crude pigment in the distillery effluent (5%) by about 75% and 60%, respectively, within 30 days of treatment [46].

Mixed culture treatment

Several researchers studied the efficiency of mixed culture microorganisms for degradation and decolorization of distillery wastewater. The mixed microbial cultures exhibited increase in mineralization of effluents over that showed by individual cultures. This might be due to the enhanced effect of coordinated metabolic interactions present in mixed community [47,48]. Bharagava et al. [49] observed enhanced growth, enzyme production and melanoidin degradation by mixed bacterial culture compared to axenic bacterial culture. In that report a mixed consortium comprised of Bacillus licheniformis, Bacillus sp. and Alcaligenes sp. showed melanoidin decolorization of about 73.79 and 69.83% for synthetic and natural melanoidins whereas axenic cultures decolorized 65.88, 62.56 and 66.10% synthetic and 52.69, 48.92 and 59.64% natural melanoidins, respectively. In another report, a mixed bacterial culture comprised of Bacillus thuringiensis, Bacillus brevis and Bacillus sp. exhibited two-to four fold increase in melanoidin decolorisation over that showed by any individual Bacillus isolate [47]. Pant and Adholeya [48] developed a novel fungal consortium comprised of Penicillium pinophilum, Alternaria gaisen, Aspergillus flavus, Fusarium verticillioides, Aspergillus niger and Pleurotus florida for decolorization of distillery effluent using agricultural residues as a growth substrate. The fungal consortium was run on a bioreactor with undiluted distillery effluent for 40 days. In the first 14 days, 61.5% color and 65.4% COD removal was achieved.

Mechanism of Melanoidin Decolorization by Microorganisms

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 laccases, 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 Ps4a [36,54], acetogenic 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 Mn²⁺, 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, laccase, 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 reposts 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

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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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