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Treatment of Distillery Waste Water: A Review

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ABSTRACT: Distilleries generating huge amount of acidic, recalcitrant and colored wastewaters with high organic content. The dark brown color of Distillery wastewater is mainly due to the high molecular weight organic compounds called melanoidin. Wastewater disposal into the environment is hazardous and has a considerable pollution potential. It reduces sunlight penetration in rivers and lakes which in turn decrease both photosynthetic activity and dissolved oxygen concentration whereas on land, it causes reduction in soil alkalinity and inhibition of seed germination. So the disposal of this liquid waste is one of the most critical environmental issues. A number of processes covering anaerobic, aerobic as well as physic-chemical methods have been employed to treat this wastewater. Within this review, presents an account of the problem and the description of color causing substances in distillery wastewater and the technologies employed globally for its treatment.

Keyword: Distillery effluent, Aerobic, Melanoidin, Pollution, Wastewater treatment, Anaerobic

I. INTRODUCTION

Molasses containing wastewater are generated by distilleries, fermentation industries, sugar mills and other molasses based industries. Molasses from sugarcane industry is it common raw material used in ethanol production due to its easy availability and low cost [1]. India is the second largest producer of ethanol in Asia. There are 319 distilleries in India with an installed capacity of 3.25 billion liters of alcohol [1-2]. The central pollution control board (CPCB) categorizes distillery industry among 17 top polluting industries in India. For every one liters of alcohol produced 10-15 l of effluent, are generated and there by a typical distillery producing ethanol from cane molasses generates nearly half million liters of effluent daily [3-4]. Approximately, 40 billion liters of effluent is generated annually in India alone for the production of 2.3 billion liters of alcohol. Distillery is one of the most highly polluting and growth oriented industries. In India with reference to the extent of water pollution and the quantity of wastewater generated. The population equivalent of distillery waste based on BOD has been reported to be high as 6.2 billion, which means that the contribution of distillery waste in India to organic pollution is approximately seven times more than the contribution by the entire population [5-7]. These contain mostly dark brown coloured recalcitrant compounds collectively termed as melanoidin polymers which are the product of maillard reaction between the amino acids and carbonyl groups present in molasses [8]. With their high biochemical chemical oxygen demand, these effluents are environmental hazards when released in water bodies they cause oxygen depletion and associated problems, and if release in soil they reduce the soil alkalinity and manganese availability, inhibit seed germination and affect vegetation. Besides causing anaesthetic discolouration of water and soil, melanoidin pigments are also toxic to microorganisms present in soil and water [9-10]. Dark brown colour of these effluents is highly resistant to microbial degradation and other biological treatment. Melanoidins have recalcitrant compounds; thus the conventional treatment methods are not effective for complete colour removal from this stream and colour can even be increase during anaerobic treatments, due to re-polymerization of compounds [11]. Anaerobic digestion of effluents produces dark brown sludge which is used as fertilizer and the coloured water are discharged after diluting them several folds with water. Thus, ultimately fresh water resource which is a precious commodity in most parts of the world is wasted. The effluent is highly coloured with an extremely high chemical oxygen demand (COD) load and contains high percentage of dissolved organic and inorganic matter. The biochemical oxygen demand (BOD) and COD, the index of its polluting character, typically range between 35,000-50,000 mg L^{-1} and 80,000-1,00,000 mg L^{-1} respectively [12]. Apart from high organic content, distillery wastewater also contains nutrients in the form of nitrogen, phosphorus and potassium that can lead to eutrophication of water bodies.

Effluent disposal even after conventional treatment is hazardous and has a high pollution potential due to the accumulation of non-biodegradable recalcitrant compounds, which are mostly coloured and in a highly complex state melanoidin have anti-oxidant properties causing toxicity to many microorganisms involved in wastewater treatment processes [13]. Lowering of pH value of the streams, increasing organic load and obnoxious smell are some of the major problems due to distillery wastewater. The distillery wastewater poses a serious threat to water quality in several regions of the country. Disposal on land is equally detrimental causing a reduction in soil alkalinity and inhibition of seed germination. In addition to pollution, increasingly stringent environmental regulations are forcing distilleries to improve existing treatment and also explore alternative methods for effluent management [6].

II. WASTEWATER CHARACTERISTICS

Distillery effluent is reported as medium to high-strength organic wastewater. Generally the effluents term as (spent wash, stillage, slop or vinasse) from molasses based distilleries are acidic and contain large amount of dark brown coloured molasses wastewater (MWW). The characteristic of the effluent depend on the raw material used [13]; also, it is estimated that 88% of the molasses constituents end up as waste [15].

In addition, cane molasses effluent contains low molecular weight compounds such as lactic acid, glycerol, ethanol and acetic acid [16]. In general, distillery effluents are acidic, have a brown colour and have a high content of organic substances that varies according to the raw material distilled e.g. wine type, lees etc. [17-20]. Distillery wastewaters are acidic and their high organic content can cause considerable environmental pollution [18-19]. The pH values of wine distillery wastewaters range from 3.5 to 5.0 (low pH), [17, 21-27], which is also toxic for many life forms. Wine distillery wastewaters were also characterized for heavy metals, *viz.* iron and zinc, metal ions such as Ca^{2+}, K^{+} and Na^{+} [26, 28-29,]. High concentrations of these constituents [29-30], plus other nutrients such as nitrate and phosphate make possible discharge of wine distillery wastewaters into water bodies problematic, causing eutrophication and other adverse environmental effects [19, 31-32].

The distillery wastewater is recalcitrant, owing to the presence of melanoidins, and contributes color to the effluent. These compounds show antioxidant properties, are inhibitory to treatment process. An average composition of sugarcane molasses based distillery effluent from India has been described [33-34]. Cane molasses also contains around 2% of a dark brown pigment called melanoidins that impart color to the effluent [1]. Melanoidins are low and high molecular weight polymers formed as one of the final products of Maillard reaction, which is a nonenzymatic browning reaction resulting from the reaction of reducing sugars and amino compounds. This reaction proceeds effectively at temperatures above 50°C and pH 4–7. The structure of melanoidins is still not well known. Only 6–7% degradation of the melanoidins is achieved in the conventional anaerobic–aerobic effluent treatment process [35]. Due to their antioxidant properties, melanoidins are toxic to many microorganisms involved in wastewater treatment [13]. Apart from melanoidins, distillery effluent contains other colorants such as phenolics, caramel and melanin. Phenolics are more pronounced in cane molasses wastewater whereas melanin is significant in beet molasses [36].

Several problems have been encountered during biological treatment of wine distillery wastewater, linked to its high toxicity and inhibition of biodegradation due to the presence of polyphenolic compounds [23], demonstrating the antibacterial activity reported in earlier literature [19]. Polyphenol concentrations in some distillery wastewater vary considerably and can range from 29-474 mg/l [17].

Table 1: Physicochemical Characteristics of distillery waste water.

Parameter	Anaerobically treated wastewater (released in field)
Electrical Conductivity (mS/cm)	33
pH	8.2
BOD5 (ppm)	5000
COD (ppm)	25,000
Total Kjeldahl nitrogen (%)	3.5
Sodium (ppm)	500
Potassium (ppm)	2500
Manganese (ppm)	259
Magnesium (ppm)	98
Zink (ppm)	273
Copper (ppm)	396
Total dissolved solids (ppm)	21,256
Total sugar (%)	2.8
Reducing sugar (%)	0.23

Source: Pant *et al*., 2006

Polyphenols are responsible for strong inhibitory effects on microbial activity, and must be removed during wastewater treatment, owing to the environmental and public health risks they pose. Humans exposed to phenol at 1300 mg/l of concentration exhibited significant increases in diarrhoea, dark urine, mouth sores and burning of the mouth [31].

III. ENVIRONMENTAL HAZARDS OF DISTILLERY WASTEWATER

The production and the characteristics of the spent wash are highly variable and dependent on the feedstock used and various aspects of the ethanol production process. Wash water used to clean the fermenters, cooling water blow down and broiler water blow down further contribute to its variability [37]. In a Distillery, sources of wastewater are stillage, fermenter and condenser cooling water and fermenter wastewater. The liquid residues during the industrial phase of the production of alcohol are: liquor, sugarcane washing water, water from the condensers and from the cleaning of the equipment, apart from other residual water. Distillery effluent has very high biological oxygen demand (BOD), chemical oxygen demand (COD) and high BOD/COD ratio. The amount of inorganic substances such as nitrogen, potassium, phosphates, calcium, sulphates is also very high. Its recalcitrant nature is due to presence of the brown polymers, melanoidins, which are formed by Maillard amino carbonyl reaction. Table 1 gives physic-chemical characteristics of distillery effluents.

These compounds have antioxidant properties, which render them toxic to many microorganisms such as those typically present in wastewater treatment processes [38]. The defiance of melanoidins to degradation is apparent from the fact that these compounds escape various stages of wastewater treatment plants and finally enters into the environment. Apart from melanoidins, the other recalcitrant compounds present in the waste are caramel, variety of sugar decomposition products, anthocyanins, tannins and different xenobiotic compounds [39]. The unpleasant odor of the effluent is due to the presence of putriciable organics like skatole, indole and other sulphur compounds [40]. The molasses effluent that is disposed in canals or rivers produces obnoxious smell [41]. Spent wash disposal into the environment is hazardous and has high pollution potential. High COD, total nitrogen and total phosphate content of the effluent may result in eutrophication of natural water bodies [5].

The highly colored components of the effluent can block out sunlight from in rivers, lakes or lagoons which in turn decrease both photosynthetic activity and dissolved oxygen concentration affecting aquatic life. Kumar *et al*. [42] evaluated the toxic effect of distillery effluent on common guppy, *Lesbistes reticulates* and observed remarkable behavioural changes with varying effluent concentration. Impact of distillery effluent on carbohydrate metabolism of fresh water fish, *C. Carpio* has studied by Ramakritinan *et al.* [43]. Saxena and Chauhan [44] investigated the influence of distillery effluent on oxygen consumption in fresh water fish, *Labeo rohita* and observed that the presence of inorganic and organic salts in the effluent interfered with the respiration in the fish. The coagulation of gill mucous decreased dissolved oxygen consumption causing asphyxiation. Matkar and Gangotri [45] observed concentration dependent toxicity of distillery effluent on the fresh water crab, *Barythephusa guerini*. Stress due to distillery effluent caused defunct respiratory processes in the fish resulting in anaerobiosis at organ level during sub lethal intoxication. Distillery effluent disposed on land is equally hazardous to the vegetation.

It is reported to inhibit seed germination, cause soil manganese deficiency and damage agricultural crops [46-47]. Raw distillery effluent is highly toxic effect on the growth and germination of *Vigna radiata* seeds even at low concentration of 5% (v/v). Leaching of protein and carbohydrates from the seeds as well as decrease in activities of important enzymes like alkaline phosphatase and ATPase was also observed [48]. Application of distillery effluent to soil without proper monitoring, perilously affects the groundwater quality by altering its physicochemical properties such as color, pH, electrical conductivity (EC), etc. due to leaching down of the organic and inorganic ions [49]. In a study conducted by Ramana *et al*. [29] the germination percent in five crops decreased with increase in concentration of the effluent. The germination was inhibited in all the five crops studied with concentration exceeding 50%.

At the same time, organic wastes contained in distillery effluent are valuable source of plant nutrients especially N, P, K and organic substrates if properly utilized [50]. For instance, distillery effluent in combination with bioamendments such as farm yard manure, rice husk and *Brassica* residues was used to improve the properties of sodic soil [51]. The use of fungi for bioconversion of distillery waste into microbial biomass or some useful metabolites has been recently reviewed by Friedrich *et al*. [52]. The end products of bioconversion are fungal biomass, ethanol, enzymes etc. and substantially purified and decolourized effluents. Recently enhanced production of oyster mushrooms (*Pleurotus* sp.) using distillery effluent as a substrate amendment have been reported [53].

IV. DISTILLERY WASTEWATER TREATMENT

Wastewater treatment methods aim at the removal of unwanted compounds in wastewater for safe discharge into environment.

This can be achieved by using physical, chemical and biological treatment of distillery effluent is either aerobic or anaerobic but in most cases a combination of both is used. Physical treatment methods such as membrane filtration processes (nano-filtration, reverse osmosis, electro-dialysis) and adsorption techniques. Chemical treatment methods such as coagulation or flocculation combined with flotation and filtration, precipitation flocculation with Fe(II)/Ca(OH)2, electro-flotation, electro-kinetic coagulation, conventional oxidation methods by oxidizing agents (ozone), irradiation or electrochemical processes etc., remove toxic material and colloidal impurities. Melanoidins, the complex bio-polymer of amino–carbolyl compounds are very recalcitrant in nature and exists extensively not only in foods but also in wastewaters released from various agro-based industries as sugarcane molasses based distillery and fermentation industries and keeping in view the hazardous nature of melanoidins, its chemical and microbial degradation has been attempted to reduce its pollution load and also to characterize its chemical structure so that better strategies could be made for its degradation and decolourization.

A. Physico-chemical Treatment technologies for distillery wastewater

Removal of melanoidin from distillery effluent has been attempted, but with limited success so far [54, 13]. Physiochemical treatment processes such as adsorption, oxidation process, coagulation and flocculation. Table 2 have been used for removal of melanoidins from treated effluent. However, these processes still have disadvantages due to the high operation cost, high consumption of chemical agent, fluctuation of color removal efficiency, high volume of solid waste produced, formation of hazardous by-products and intensive energy requirements.

Adsorption. Activated carbon is a well known adsorbent due to its extended surface area, microporus structure, high adsorption capacity and high degree of surface reactively. Among the physicochemical treatment methods, adsorption on activated carbon is widely employed for removal of color and specific organic pollutant. Bernardo *et al*. [55-56] investigated decolourization of synthetic melanoidin using commercially available activated carbon as well as activated carbon produced from sugarcane bagasse. The adsorptive capacity of the different activated carbons was found to be quite comparable. Chemically modified bagasse using 2-diethylaminoethyl chloride hydrochloride and 3-chloro-2-hydroxypropyltrimethyl ammonium chloride was capable of decolorizing diluted effluent [57]. 0.6 g of chemically modified bagasse in contact with 100 ml 1:4 (v/v) effluent: water solution resulted in 50% decolourization after 4 h contact with intermittent swirling. Significant decolourization was observed in packed bed studies on anaerobically treated effluent using commercial activated charcoal with a surface area of 1400m^2/g [58]. Almost complete decolourization (>99%) was obtained with 70% of the eluted sample, which also displayed over 90% BOD and COD removal. In contrast, other workers have reported adsorption by activated carbon to be ineffective in the treatment of distillery effluent [59-60]. Adsorption by commercially available powered activated carbons resulted in only 18% color removal, combined treatment using coagulation-flocculation with polyelectolyte followed by adsorption resulted in almost complete decolourization [59]. Low cost adsorbents such as pyorchar (activated carbon both in granular and powdered form, manufactured from paper mill sludge) and bagasse flyash have also been studied for this application. Ramteke *et al*. [61] reported color removal up to 98% with pyorchar.

However, to achieve the same level of color removal, larger doses of the indigenously prepared powdered and granular pyorchar were required in comparison to commercial activated carbon. Mall and Kumar [14] compared the color removal using commercial activated carbon and bagasse flyash. 58% color removal was reported with 30 g/l of bagasse flyash and 80.7% with 20 g/l of commercial activated carbon. Since the bagasse flyash has high carbon content and the adsorbed organic material further increases its heating value, the spent adsorbent can be used for making fire briquettes. Yet another adsorbent that has been examined is the natural carbohydrate polymer chitosan derived from the exoskeleton of crustaceans. Lalov *et al*. [62] studied the treatment of distillery wastewater using chitosan as an anion exchanger. At an optimum dosage of 10 g/l and 30 min contact time, 98% color and 99% COD removal was observed.

Coagulation and flocculation. Coagulation is the destabilization of colloids by neutralizing the forces that keep apart. The optimum dosage of lime was found to be 10 g/l resulting in 82.5% COD removal and 67.6% reduction in color in a 30 min period. These findings are in disagreement with those of Migo *et al*. [63] used a commercial inorganic flocculent, a polymer of ferric hydroxysulfate for the treatment of molasses wastewater. The treatment resulted in around 87% decolourization for biodigested effluents; however, an excess of flocculent hindered the process due to increase in turbidity and total organic carbon (TOC) content. $FeCl_3$ and $AlCl_3$ were also tested for decolourization of biodigested effluent and showed similar removal efficiencies. About 93% reduction in color and 76% reduction in TOC were achieved when either $FeCl_3$ or $AlCl_3$ was used alone. The process was independent of chloride and sulfate ion concentration but was adversely affected by high fluoride concentration.

Table 2: Physicochemical methods employed for distillery wastewater treatment.

Treatment	% COD removal	% Color removal	Reference
Adsorption			
Chitosan, a biopolymer was used as anion exchanger	99	98	[59]
Chemically modified bagasse			
DEAE bagasse	40	51	[54]
CHPTAC bagasse	25	50	
Activated carbon prepared from agro industrial waste			
Phosphoric acid carbonized bagasse was used	23	50	[10]
Commercially available activated carbon			
AC (ME)	76	93	
AC (LE)	88	95	
Coagulation-flocculation			
Flocculation of synthetic melanoidins was carried out by various inorganic ions			
Polyferric hydroxysulphate (PFS)	NR	95	
Ferric chloride (FeCl3)	NR	96	
Ferric sulphate (Fe2 $(SO_4)3$)	NR	95	
Aluminium sulphate (Al2(SO4)3)	NR	83	[60]
Calcium oxide (CaO)	NR	77	
Calcium chloride ($CaCl_2$)	NR	46	
Different inorganic ions and waste water from Iron pickling and Titanium process industry were used as coagulants. Addition of polyelectrolyte percol 47 reduced their dosage			
Ferrous sulphate ($FeSO_4$)	78	98	
Ferric sulphate (Fe2 $(SO4)_3$)	77	96	
Alum	64	95	[38]
Iron pickling waste water	86	99	
Titanium processing waste water	67	99	
Iron chloride coagulation	38	47	[61]
Iron chloride	65	69	
Aluminium chloride	61.3	74.4	[62]
Calcium oxide (CaO)	39.8	80.2	
Ferric chloride ($FeCl_3$)	55	83	[63]
Aluminium chloride ($AlCl_3$)	60	86	
Polyaluminium (PAC)	72	92	
Oxidation processes			
Fenton's oxidation	88	99	[64]
Ozonation	15-25	80	[65]
Elecrochemical oxidation			
Graphite electrodes	80.6	95.6	
Lead dioxide coated on titanium	90.8	98.5	[66]
Ruthedium dioxide coated on titanium	92.1	99.5	
Electrocoagulation and electro Fenton	92.6		[67]
Membrane technologies			
Reverse osmosis	99.9		[27]
Nanofiltration	97.1	100	

However in the presence of high flocculent concentration (40 g/l), addition of 30 g/l CaO enhanced the decolourization process resulting in 93% color removal. This was attributed to the ability of calcium ions to destabilize the negatively charged melanoidins; further, formation of calcium fluoride (CaF_2) also precipitates the fluoride ions.

Almost complete color removal (98%) of biologically treated distillery effluent has been reported with conventional coagulants such as ferrous sulfate, ferric sulfate and alum under alkaline conditions [39]. The best results were obtained using Percol 47, a commercial organic anionic polyelectrolyte, in combination with ferrous sulfate and lime. The combination resulted in 99% reduction in color and 87 and 92% reduction in COD and BOD, respectively. Similar findings have also been reported by Mandal *et al.* [60]. Coagulation studies on distillery effluent after anaerobic–aerobic treatment have also been conducted using bleaching powder followed by aluminum sulfate [71].

The optimum dosage was 5 g/l bleaching powder followed by 3 g/l of aluminum sulfate that resulted in 96% removal in color, accompanied by up to 97% reduction in BOD and COD. Non-conventional coagulants namely wastewater from an iron pickling industry which is rich in iron and chloride ions and titanium ore processing industry containing significant amounts of iron and sulfate ions have also been examined [39]. The iron pickling wastewater gave better results with 92% COD removal, combined with over 98% color removal. Though the titanium processing wastewater exhibited similar color removal levels, the COD and BOD reductions were perceptibly lower.

Oxidation process. Ozone destroys hazardous organic contaminants and has been applied for the treatment of dyes, phenolics, pesticides, etc. [68]. Oxidation by ozone could achieve 80% decolourization for biologically treated effluent with simultaneous 15–25% COD reduction. It also resulted in improved biodegradability of the effluent. However, ozone only transforms the chromophore groups but does not degrade the dark colored polymeric compounds in the effluent [68, 72]. Similarly, oxidation of the effluent with chlorine resulted in >97% color removal but the color reappeared after a few days [60]. Ozone in combination with UV radiation enhanced spent wash degradation in terms of COD; however, ozone with hydrogen peroxide showed only marginal reduction even on a very dilute effluent [73]. In another study, Sangave and Pandit [74] employed sonication of distillery wastewater as a pre-treatment step to convert complex molecules into a more utilizable form by cavitation. Samples exposed to 2 h ultrasound pretreatment displayed 44% COD removal after 72 h of aerobic oxidation compared to 25% COD reduction shown by untreated samples. These results are contrary to those of Mandal *et al*. [60] who concluded ultrasonic treatment to be ineffective for distillery effluent treatment.

A combination of wet air oxidation and adsorption has been successfully used to demonstrate the removal of sulfates from distillery wastewater. Studies were done in a counter current reactor containing 25 cm base of small crushed stones supporting a 20 cm column of bagasse ash as an adsorbent [75]. The wastewater was applied from the top of the reactor and air was supplied at the rate of 1.0 l/min. The treatment removed commercially available powdered activated carbons resulted in only 18% color removal; however, combined treatment using coagulation–flocculation with polyelectrolyte followed 57% COD, 72% BOD, 83% TOC and 94% sulfates. Wet air oxidation has been recommended as part of a combined process scheme for treating an-aerobically digested spent wash [76]. The post-anaerobic effluent was thermally pre-treated at 150°C under pressure in the absence of air. This was followed by soda-lime treatment, after which the effluent underwent a 2 h wet oxidation at 225°C. 95% color removal was obtained in this scheme. Another option is photo catalytic oxidation that has been studied using solar radiation and TiO_2 as the photo catalyst [77]. Use of TiO_2 was found to be very effective as the destructive oxidation process leads to complete mineralization of effluent to CO_2 and H_2O. Up to 97% degradation of organic contaminants was achieved in 90 min. Pikaev *et al*. [78] studied combined electron beam and coagulation treatment of distillery slops from distilleries processing grain, potato, beet and some other plant materials. Humic compounds and lignin derivatives constitute the major portion of this dark brown wastewater. The distillery wastewater was diluted with municipal wastewater in the ratio of 3:4, irradiated with electron beam and then coagulated with $Fe_2(SO_4)_3$. The optical absorption in UV region was decreased by 65–70% after this treatment. The cost was found to be less than the existing method wherein the effluent was transported about 20 km via pipeline to a facility for biological treatment followed by sedimentation. The treatment cost was 0.45–0.65 US$/m^3 which dropped to 0.25 US$/m^3 using combined electronic-beam and coagulation method.

Other treatments. Pikaev *et al*. [78] applied radiation technology for treatment of distillery waste. The study involved a combined treatment of electron beam (dose 20 kGy) and coagulation using $Fe_2(SO)_3$ which resulted in a decrease in optical absorption in the *uv* region by 65–70% in the treated effluent. Ultrasound technology was also applied for the treatment of distillery effluent. Studies were carried out to find out the efficacy of the ultrasonic irradiation as a pre-treatment step and the results indicated that ultrasound treatment enhanced the biodegradability of the distillery waste water [74]. Chaudhari *et al*. [79] proposed a novel catalytic thermal pre-treatment or catalytic thermolysis (CT) to recover the majority of its energy content with consequent COD and BOD removal. They found that the initial pH (pH) had profound impact on the efficiency of thermolysis in COD removal. At 140°C with 3kgm^{-3} catalyst loading and pH 2 (optimum value), they observed a maximum of 60% COD removal. The CT process resulted in the formation of settleable solid residue and the slurry obtained after the thermolysis exhibited very good filtration characteristics. At 140°C and pH 2, the solid residue had a C:H atomic ratio of 1:1.08 with a heating value of 21.77MJ kg^{-1}. The residue can be used as a fuel in the combustion furnaces and the ash obtained can be blended with organic manure and used in agriculture/horticulture. Kannan *et al.* [80] adopted electro coagulation technique with addition of indigenously prepared areca nut carbon (AAC) for treatment of distillery effluent. This study, for a period of 1 h, resulted in almost colourless effluent with 89.7% BOD and 80% COD removal. This process resulted in the formation of settleable solid residue and the slurry obtained after the thermolysis exhibited very good filtration.

It can be used as a fuel in the combustion furnaces and the ash obtained can be blended with organic manure and used in agriculture/horticulture. Various physico-chemical methods such as adsorption, coagulation-flocculation and oxidation processes like Fenton's oxidation, ozonation, electrochemical oxidation using various electrodes and electrolytes, nonofiltration, reverse osmosis, ultrasound and different combinations of these methods have also been tested for the treatment of distillery effluent. As mentioned above, sugarcane molasses wastewaters have been reported to be decolorized by various physico-chemical methods which are summarized above.

Physico-chemical treatment methods are effective in both color and COD removal. Nevertheless the drawbacks associated with these methods are excess use of chemicals, sludge generation with subsequent disposal problems, high operational cost and sensitivity to variable water input [81]. Considering the advantages and the disadvantages of different treatment technologies, no single technology can be used for complete treatment of molasses wastewater. Hence, there is a need to establish a comprehensive treatment approach involving several technologies sequentially.

B. Biological/microbial Treatment

Microorganisms due to their inherent capacity to metabolize a variety of substrate have been utilized since long back for biodegradation of complex, toxic and recalcitrant compounds which cause severe damage to environment. Thus, these organisms have been exploited for biodegradation and decolourization of melanoidin pigment present in industrial wastes especially from distillery and fermentation industry.

Anaerobic treatment. The high organic content of molasses wastewater makes anaerobic treatment attractive in comparison to direct aerobic treatment. Anaerobic digestion is viewed as a complex ecosystem in which physiologically diverse groups of microorganisms operate and interact with each other in a symbiotic, synergistic, competitive or antagonistic association. In the process methane and carbon dioxide are generated [82]. Molasses wastewater treatment using anaerobic process is a very promising re-emerging technology which presents interesting advantages as compared to classical aerobic treatment. It produces very little sludge, requires less energy and can be successfully operated at high organic loading rates; also, the biogas thus generated can be utilized for steam generation in the boilers thereby meeting the energy demands of the unit [83]. Further, low nutrient requirements and stabilized sludge production are other associated benefits [84]. However, the performance and treatment efficiency of anaerobic process can be influenced both by inoculum source and feed pretreatment. These processes have been sensitive to organic shock loadings, low pH and showed slow growth rate of anaerobic microbes resulting in longer hydraulic retention times (HRT). This often results in poor performance of conventional mixed reactors. In order to solve these problems, several high rate configurations have been developed for treating soluble wastewater at relatively shorter HRTs [85].

Anaerobic lagoons are the simplest choice for anaerobic treatment of molasses wastewater. Rao [86] carried out the pioneering research work in the field of distillery waste management by employing two anaerobic lagoons in series, resulting in BOD removal ranging from 82 to 92%. However, the lagoon systems are seldom operational, souring being a frequent phenomenon. Large area requirement, odor problem and chances of ground water pollution are drawbacks [87].

The conventional digesters such as continuous stirred tank reactors (CSTR) are the simplest form of closed reactors with gas collection. Treatment of molasses wastewater in CSTR has been reported in single as well as biphasic operations, resulting in 80–90% COD reduction within a period of 10–15 days [88]. Treatment of distillery waste using batch reactors has not been widely attempted. Treatment of winery wastewater was investigated using an anaerobic sequencing batch reactor (ASBR). The reactor was operated at an OLR of 8.6 kg COD m^{-3} d^{-1} with soluble COD removal efficiency greater than 98% with HRT of 2.2 days [89]. Table 3 summaries the functioning of various anaerobic reactors covering both laboratory studies and profitable operations for treatment of alcohols based distilleries wastewater.

Banerjee and Biswas [90] designed a semi-continuous batch digester to investigate biomethanation of distillery waste in mesophilic and thermophilic range of temperatures. The study revealed that there was an important effect of the temperature of digestion and of substrate concentration in terms of BOD and COD loading on the yield of biogas as well as its methane content. Maximum BOD reduction (86.01%), total gas production and methane production (73.23%) occurred at a BOD loading rate of 2.74 kg m^{-3} at 50°C digestion temperature.

In fixed film reactors, the reactor has a biofilm support structure (media) for biomass attachment. Fixed film reactor offers the advantages of simplicity of construction, elimination of mechanical mixing, better stability even at higher loading rates and capability to withstand toxic shock loads. The reactors can recover very quickly after a period of starvation [25, 91]. In another study, Perez-Garcia *et al*. [92] studied the influent pH conditions in fixed film reactors for anaerobic thermophilic treatment of wine distillery wastewaters.

Table 3: Anerobic methods employed for distillery wastewater treatment.

Reactor type	Organic loading rate (OLR)(kgCOD m^{-3} day -1)	COD removal (%)	BOD Removal (%)	Retention time (Days)	Reference
Downflow fixed-film reactor	-	60-73	85-97	-	[89]
Granular bed Anaerobic baffled reactor	2.4	90-96	80-92	4	[90]
Hybrid anaerobic baffled reactor	20	70	-	-	[91]
Upflow anaerobic sludge blanket (UASB) reactor	28	39-67	80	-	[25]
Istanbul UASB reactor	6-11	90	-	-	[92]
Tekirdag UASB reactor	2.5-8.5	60-80	-	-	
Upflow anaerobic sludge blanket at Tekirdag (TUASB)	2.5-8.5	60-80	-	-	[93]
Upflow anaerobic sludge blanket at (Istanbul)	1-4.5	70-80	-	-	
Diphasic fixed-film reactor with granular activated carbon (GAC) as support media	21.3	67.1	-	4	[94]
Anaerobic contact filter	19,000mg L-1 (influent COD concentration)	73-98	-	4	[95]
UASB	24	75	-	-	[96]
UASB[a]	15	90	-	2.1	[97]
UASB[b]	18	>90	-	-	[21]
Thermophilic UASB	Up to 86.4	60	-	-	[98]
Thermophilic UASB	Up to 30	87	-	0.3	[99]
Two-stage anaerobic treatment[a] Anaerobic filter UASB	2.5-5.1 0.6-2.5	54 93		10-19 20-39	[100]
Upflow blanket filter	9-11	70		11-12	[101]
Upflow anaerobic filter (UAF)[a]	20	76			[102]
Two-stage bioreactor (anaerobic) Ist stage (upflow sludge bed reactor) IInd stage (batch operated bioreactor, flocculator, precipirator)	7		71	86 11 0.10	[20]
Downflow fluidized bed reactor with ground perlite[b]	17 kg TOC/m^3d	75-95% TOC		0.35	[103]
Downflow fluidized bed reactor with ground perlite[b]	4.5	85		3.3-1.3	[104]
Downflow filter	8	55-85			[105]
Two-phase thermophilic process Acidogenesis Methanogenesis	4.6-20.0	65	85	2 15.2	[106]
Diphasic (upflow) fixed film reactor (clay brick granules support)	22	71.8		3	[107]

The Upflow anaerobic sludge blanket (UASB) process has been successfully used for the treatment of various types of wastewaters [112]. UASB reactor systems belong to the category of high rate anaerobic wastewater treatment and hence it is one of the most popular and extensively used reactor designs for treatment of distillery wastewaters globally. The success of UASB depends on the formation of active and settleable granules [113-115]. In anaerobic fluidized bed reactor (AFB), the medium which support bacteria attachment and growth is kept in the fluid state by drag forces exerted by the up flowing wastewater. The media used are small particle size sand, activated carbon, etc. In the fluidized state, each medium provides a large surface area for biofilm formation and growth. It enables the attainment of high reactor biomass hold-up and promotes system efficiency and stably. Kida *et al*. [116] studied the biological treatment of Shochu distillery wastewater using an anaerobic fluidized bed reactor.

Aerobic treatment. Anaerobically treated distillery wastewater still contains high concentrations of organic pollutants and then cannot be discharged directly. The partially treated spent wash has high BOD, COD and suspended solids. It can reduce the availability of essential mineral nutrients by trapping them into immobilized organic forms, and may produce phytotoxic substances during decomposition. Stringent regulations on discharge of colored effluent impede direct discharge of anaerobically treated effluent [83]. Therefore, aerobic treatment of sugarcane molasses wastewater has been mainly attempted for the decolourization of the major colorant, melanoidins, and for reduction of the COD and BOD. A large number of microorganisms such as bacteria (pure and mixed culture), cyanobacteria, yeast and fungi have been isolated in recent years and are capable of degrading melanoidins and thus decolorizing the molasses wastewater. The aerobic methods have been described below.

Activated sludge process. The most common wastewater treatment is the activated sludge process where in research efforts are targeted at improvements in the reactor configuration and performance. For instance, aerobic sequencing batch reactor (SBR) was reported to be a promising solution for the treatment of effluents originating from small wineries [117]. The treatment system consisted of a primary settling tank, an intermediate retention trough, two storage tanks and an aerobic treatment tank. A start up period of 7 days was given to the aerobic reactor and the system resulted in 93% COD and 97.5% BOD removal. The activated sludge process and its variations utilize mixed cultures. To enhance the efficiency of aerobics systems, several workers have focused on the treatment by pure cultures. Though aerobic treatment like the conventional activated sludge process is presently practiced by various molasses-based distilleries and leads to significant reduction in COD, the process is energy demanding and the color removal is still unsatisfactory.

Biocomposting is a method of activated bioconversion through the aerobic pathway, whereby heterotrophic microorganisms act on carbonaceous materials depending on the availability of the organic source and the presence of inorganic materials essential for their growth. Composting is particularly effective in converting the wet materials to a usable form thereby stabilizing the organic materials and destroying the pathogenic organisms in addition to significant drying of the wet substrates. In the composting process, under aerobic conditions, thermophilic biodegradation of organic wastes at 40-60% moisture content occurs to form relatively stable, humus-like materials [48].

Phytoremediation. Phytoremediation of effluents is an emerging low cost technique for removal of toxicants including metals from industrial effluents and is still in an experimental stage. Aquatic plants have excellent capacity to reduce the level of toxic metals, BOD and total solids from the wastewaters [118-119]. Billore *et al*. [120] carried out the treatment of distillery effluent in a constructed wetland which comprised of four cells. After a pretreatment in the two first cells the effluent was channeled to cells three and four which contained plants *Typha latipholia* and *Phragmites karka*. This treatment eventually led to 64% COD, 85% BOD, 42% total solids and 79% phosphorus content reduction. Kumar and Chandra [118] successfully treated distillery effluent in a two stage process involving transformation of recalcitrant coloring components of the effluent by a bacterium *Bacillus thuringienesis* followed by subsequent reduction of remaining load of pollutants by a macrophyte *Spirodela polyrrhiza*. A similar biphasic treatment of the effluent was carried out in a constructed wetland with *Bacillus thuringienesis* and *Typha angustata* by Chandra *et al*. [121] which resulted in 98– 99% BOD, COD and color reduction after 7 days. Phytoremediaion of effluents is an emerging lowcost technique for removal of toxicants including metals from industrial effluents and is still in an experimental stage [122]. Cyanobacteria are considered ideal for treatment of distillery effluent as they, apart from degrading the polymers also oxygenate water bodies, thus reduce the BOD and COD levels. Marine *cyanobacteria* such as *Oscillatoria boryna* have also been reported to degrade melanoidins due to the production of H_2O_2, hydroxyl, per hydroxyl and active oxygen radicals, resulting in the decolourization of the effluent [1]. Patel *et al*. [123] have reported 96%, 81% and 26% decolourization of distillery effluent through bioflocculation by *Oscillatoria* sp*., Lyngbya* sp. and *Synechocystis* sp., respectively.

Valderrama *et al.* [124] studied the feasibility of combining microalgae, *Chlorella vulgaris* and macrophyte *Lemna minuscule* for bioremediation of wastewater from ethanol producing units. This combination resulted in 61% COD reduction and 52% color reduction. First, the microalgal treatment led to removal of organic matter and further treatment with macrophytes removed other organic matter, color and precipitated the microalgae.

Fungal treatment of distillery wastewater. Fungi, due to their characteristic morphology (i.e. developed hyphae/ mycelium) have excellent adsorption property. These also possess well developed enzymatic system to breakdown complex substrates to derive metabolic energy. Due to such unique features, fungi have been widely exploited for the degradation and decolourization of melanoidin containing wastewater. Watanabe *et al.* [125] obtained significant melanoidin decolourising activity (MDA) with *Coriolus* sp. They reported a decreased of 77% in darkness of melanoidin solution (0.5% v/v) under the culture conditions at 30°C for two weeks.

Similarly, Aoshima *et al.* [126] had screened about 23 genera, 30 strains belonging to white and brown rot fungi for melanoidin degradation and recorded grater variation in melanoidin decolourization activity in various white-rot fungi e.g. *Coriolus hirsutus, Coriolus versicolor* Ps4a, *Fomitopsis, Cystisina, Irpex lacteus* Ps8a*, Lenzites betulina* L5b etc. According to them *Coriolus versicolor* Ps4a showed highest activity, a decolourization yield of approximately 80% under the optimal conditions. They also reported that production of MDA by *C. versicolor* was almost completely coincident with the growth of mycelia and was mainly due to intracellular enzymes and induced by the molasses melanoidin pigment (Table 4). Ohmomo *et al.* [127] had studied the continuous decolourization of molasses waste treated by means of methane fermentation and activated sludge with the mycelia of *Coriolus versicolor* Ps4a under both free-cell and immobilized conditions. They attained a decolourization yield of approximately 75% in a bubbling column reactor and found that optimum decolourization with bare pellet-type mycelia in shaking flasks needed the addition of external carbon (glucose, 0.5%w/v) and nitrogen [peptone, 0.05% (w/v)] and aerobic conditions (1 ppm of dissolved oxygen). Their studies on waste water decolourization with mycelia immobilized within calcium alginate gel in a bubbling column reactor under the optimum conditions yielded constant decolourization (65.7%) during continuous decolourization for 16-days. They also stressed the need of added glucose and peptone to be necessary to maintain the (melanoidin degrading activity) MDA of mycelia.

Later, they have reported the products of enzymatic decolourization of melanoidin using crude enzymes isolated and purified from mycelia of *Coriolus versicolor* Ps4a. They obtained some amino acids and organic acids from decolourised fraction of melanoidin. The organic acid fraction from glucose-glycine melanoidin (GGM) gave lactic acid as major acid and formate, oxalate etc. as minor acids. The amount of lactic acid was equivalent to 10.5% of the carbon in melanoidin. Ohmomo *et al*. [128] screened fungi that were found able to decolourise molasses melanoidin in the tropical zone and isolated some strains mainly of genus *Aspergillus*. They recovered a strain no. G-2-6 most active, thermophilic and identical with *Aspergillus fumigatus. A. fumigatus* (G-2-6) decolourised about 75% of molasses melanoidin pigment when it was cultivated on a glycerol peptone medium at 45°C for 3-days under shaking conditions. They also investigated the continuous decolourization of molasses melanoidin pigment in a jar fermentor and found that it had an almost constant decolourization yield of about 70%.

At the same time, they observed the decrease of about 51% in chemical oxygen demand (COD) and 56% removal of the total organic carbon (TOC) in initial solution. In contrast, they reported continuous decolourization of non-dialyzed molasses melanoidin removed a little more COD and TOC than that of dialyzed molasses melanoidin but had a lower level of melanoidin decolourising activity (about 40%). Ohmomo *et al.* [128] have also studied the molasses melanoidin decolourization by using the different strains of *Aspergillus oryzae* (eg. *A. oryzae* IAM 2731, *A. oryzae* IFO 5786 and *A. oryzae* Y-2-32) grown on glycerol-peptone medium and found that *A. oryzae* Y-2-32 was most active which decolourised about 75% melanoidin when cultivated at 35°C for 4-days under shaking conditions. They have also observed that the type of sugars utilized for growth influenced the degree of adsorption which melanoidin-adsorbing ability of mycelia was repressed by a high concentration of salt (e.g. NaCl or buffer).

Sirianuntapiboon *et al.* [152] screened 228 strains of filamentous fungi belonging to classes Deuteromycetes and Basidiomycetes possessing the ability to degrade melanoidin containing molasses effluent. They found that strain D-90 has shown the highest decolourization potential (~93%) when it was cultivated at 30°C for 8-days in molasses solution containing (w/v): glucose (2.5%), yeast extract (0.2%) and inorganic salts (KH_2PO_4 0.1% and $MgSO_4.7H_2O$ (0.05%). The strain D-90 was later found to be identical with the order *Mycelia sterilia.*

Table 4: Fungi employed for the decolourization of distillery wastewater.

S. No.	Name	Comments	Colour removal (%)	Reference
1.	*Phanerochaete Chrisosporium*	Both the fungi required a readily avalaible carbon source for melanoidin and decolourisation while N source had no effect maximum dicoulourisation was observed in 6.25 % (v/v) spent wash	53.5	[124]
2.	*Coriolus versiculor*		71.5	
3.	*Trametes versiculor*	COD and N- NH_4 removal observed in presence of sucrose and KH_2PO_4 as nutrient source.	82	[125]
4.	*Geotrichum candidum*	Fungus immobilized on polyurethane form showed stable decolourisation of molasses in repeated-batch cultivation	80	[126]
5.	*Coriolus hirsutus*	A large amount of glucose was required for colour removal but addition of peptone reduced the decolourising ability of the fungus.	80	[127]
6.	*Penicillium* sp.	All fungi produced decolourisation from first day of incubation, with maximum being shown by P. decumbens at fourth day with a reduction of 70% of the phenolic content of the waste water.	30	[128]
7.	*Penicillium decumbens*		41	
8.	*Penicillium lignorum*		28	
9.	*Aspergillus niger*		25	
10.	*Aspergillus niger UM2*	Decolourisation was more immobilized fungus and it was able to decolorize upto 50% of initial effluent concentrations.	80	[129]
11.	*Aspergillus fumigates G-2-6*	Thermophilic strain tried for molasses waste water decolourisation but colouring compounds hardly degraded.	56	[123]
12.	*Mycelia strilia*	Organism required glucose for the decoloursing activity.	93	[130]
13.	*Aspergillus niger*	Maximum colour removal was obtained when $MgSO_4$, KH_2PO_4, NH_4NO_3 and a carbon source was added to waste water.	69	[131]
14.	*Flavodon flavus*	MSW was decolourised using a marine basidiomycete fungus. It also removed 68% benzo(a) pyrene, a PAH found in MSW.	80	[33, 132]
15.	*Rhizoctonia* sp. *D-96*	Mechanism of decolourisation of melanoidin involved absorbtion of the melanoidin pigment by the cells as a macro molecule and its intracellular accumulation in the cytoplasm and around the cell membrane as a melanoidin complex, which was then gradually decolourised by intracellular enzymes	90	[133]
16.	*Coriolus varsicolor Ps4a*	Two types of enzymes, sugar dependent and sugar independent, were found to be responsible for melanoidin decolourising activity	80	[122]
17.	*Aspergillus oryzae Y-2-32*	The thermophilic strain adsorbed lower molecular weight fractions of melanoidin and required sugar for growth.	75	[134]
18.	*Phanerochaete chrysosporium JAG-40*	This organism decolourised synthetic and natural melanoidins when the medium was supplemented with glucose and pepton	80	[135]
19.	*Coriolus hirsutus IFO4917*	Melanoidin present in heat treatment liquor were subjected to sequencing batch decolourisation by the immobilized fungal cells	45	[136]
20.	*Aspergillus niveus*	The fungus could use sugarcane bagasse as carbon source and required other nutrient for decolourisation.	56	[137]
21.	*Trametes* sp. *I-62*	No colour observed associated with either fungal mycelium or polysaccharides secreted by the fungus and therefore colour removal were attributed to fungal dregradation and not to a simple physical binding.	73	[34]

S. No.	Name	Comments	Colour removal (%)	Reference
22.	*Aspergillus niger*	All these organisms were isolated from an air bubble column reactor treating winery waste water after 6 months of operation	Not checked in this study	[138]
23.	*Candia* sp.			
24.	*C. lambica*			
25.	*C. lypolitica*			
26.	*Fusarium* sp.			
27.	*Penicillium* sp.			
28.	*P. roquefortii*			
29.	*Saccharomyces cerevisiae*			
30.	*Trichoderma koningii*			
31.	*Coriolus* sp. *no. 20*	First strain for the application of its ability to remove melanoidins from MWW, showed decolourisation activity in 0.5% melanoidin when sorbose or glucose was added as carbon source.	80	[120]
32.	*Williopsis saturnus strain CBS 5761*	Yeast isolates from a rotating biological contactor (RBC) treating winery waste water. Only 43% COD removal could be achieved.	Not checked in this study	[139]
33.	*Pichia membranaefaciens strain IGC 5003*			
34.	*Candia intermedia JCM 1607*			
35.	*Erumothecium gossyphi*			
36.	*Saccharomyces cerevisiae starin J2*			
37.	*Hanseniaspora uvarum*			
38.	*Coriolus versicolor* sp. *no. 20*	10% diluted spent wash was used with glucose @ 2% added as carbon source.	34.5	[140]
39.	*Phanerochaete*	Sugar refinery effluent was treated in a RBC using polyurethane foam chrysosporium and scouring web as support.	55	[141]
40.	*Pycnoporus coccineus*	Immobilized mycelia removed 50% more colour then free mycelia.	60	[142]
41.	*Coriolus versicolor*	Cotton stalks were added as additional carbon source which stimulated the decolourisation activity of all fungi in 30% vinasses.	63	[143]
42.	*Phanerochaete chrysosporium*		37	
43.	*Funalia trogii*		57	
44.	*Pleurotus pulmonarius*		43	
45.	*Aspergillus-UB2*	This was with diluted waste water with optimum values of supplemented materials.	75	[144]
46.	Marine vasidiomycete NIOCC # 2a		100	[145]
47.	*Phanerochaete chrysosporium* NCIM 1073 0 NCIM 1106 82 NCIM 1197 76	Molasses medium decolorsation was checked in stationary and submerged cultivation conditions.		[146]
48.	*Citromyces* sp. *WR-43-6*	Organism required glucose, sodium nitrate and KH_2PO_4 for maximal decolourisation.	68.91	[147]
49.	*Hansenula fabianii*	The flocculant strains could reduce 28.5% TOC from base water without dilution.	Not checked in this study	[148]
50.	*Hansenula anomala*			

Further, they carried out detailed studies on the decolourization of molasses wastewater by *Mycelia sterilia* D-90 and reported 90% decolourization of molasses pigment in 10-days. These workers have also demonstrated a simultaneous decrease of 80% in biological oxygen demand (BOD) by supplementing (w/v): glucose 2.5%, $NaNO_3$ 0.2%, KH_2PO_4 0.1% and $MgSO_4$ $7H_2O$ 0.05% as nutrient. They reported that decolourization yield was 17.5% in absence of nutrient. Furthermore, *Mycelia sterilia* (D-90) showed the decolourization yield of about 70% in 11 days and caused a decrease in BOD value of about 90% in 15 days under non-sterile conditions. In fed batch system the same strain showed constant decolourization yield of about 80% and caused a decrease in BOD value of about 70% during three times replacement (24 days).

Fahy *et al.* [154] have reported the decolourization of molasses effluent (MSW) by *Phanerochaete chrysosporium* ATCC24725 and stated that *P. Chrysosporium* decolourised molasses effluent (6.25%, v/v) supplemented with glucose (2.5% w/v) about 85% after 10-days incubation. In presence of both carbon (glucose) and nitrogen (peptone) sources the decolourization yield decreased upto 21%. They have also investigated MSW decolourization under immobilized conditions using calcium alginate gel and reported that immobilized cells decolourised MSW more rapidly than free cells but there was an overall colour decrease (59%) after 10 days. Later, they reported the microbial decolourization of melanoidin containing wastewater by using the fungus *Coriolus hirsutus* along with activated sludge. They pre-treatment of heat treatment liquor (HTL) a melanoidin containing wastewater by activated sludge could enhance the fungal decolourization of HTL by two to three folds. These researches have also defined the role of Mn on HTL decolourization as well as peroxidase production during HTL decolourization.

Bacterial treatment of distillery wastewater. The reports on the decolourization of melanoidin polymer by bacterial strains are very recent. Due to versatility in the nature of nutrient utilisation, the bacteria are capable to degrade different xenobiotic compounds including melanoidin polymer. Ohmomo *et al.* [139] screened some facultative anaerobes with melanoidin decolourising activity (MDA). They reported that strain W-NS showed high and stable MDA and was identical to *Lactobacillus hilgardii*. The decolourization yield of this strain under optimum conditions was 28%. However, the immobilization of cells within Calcium alginate gels improved the decolourization yield to 40%. According to these researchers unlike Ascomycetes and Basidiomycetes, this strain decolourised smaller molecular weight fractions of melanoidins quickly. They also reported the MDA of this strain towards various synthetic melanoidins (e.g. from glucose and glycine; glucose and valine etc.). The MDA of this strain was found quiet different from that of Basidiomycetes. Investigations on the continuous decolourization of molasses wastewater (MWW) by using the immobilized *Lactobacillus hilgardii* W-NS cells have shown the maximal decolourization yield (90%) in presence of glucose (1% w/v) at 45°C. Further, the successive decolourization of MWW with the recycling of immobilized cells was recorded more than 90% of the maximal decolourization that was maintained for one month when peptone (0.05%) was added to MWW. However, on adjusting the medium pH to neutral (pH 7.3) compared with the maximal pH value (5.0) has slowed down the decrease in the decolourization yield. Kumar *et al*. [5, 38] reported that two aerobic bacterial cultures LA1 and D-2 showed the highest decolourization (36.5 and 32.5%) and COD reduction (41 and 39%) respectively under optimum conditions. They suggested that the decolourization achieved might be due to the degradation of smaller molecular weight fractions of melanoidin. These investigations have ruled out the possible involvement of manganese dependent peroxidase and all other lignolytic peroxidases as suggested by previous workers in the decolourization of melanoidin containing molasses spent wash. They suggested that decolourization may have occurred as a result of secondary metabolic reaction resulting from a secondary metabolite. Nevertheless, the actual mechanism of melanoidin degradation according to them remains yet to be confirmed. A facultative anaerobic bacterial strain L-2 belonging to genus *Lactobacillus* showed high decolourization activity. It achieved 31% decolourization and 57% COD reduction after 7-days incubation at 37°C in (12.5% v/v) diluted spent wash.

Nakajima-Kambe *et al.* [155] screened number of microorganisms and selected a strain (MD-32) of the genus *Bacillus*, with very high potential for decolourised molasses pigment upto 35.5% within 20-days at 55°C under anaerobic conditions but showed no decolourization activity when aerobically cultivated (Table 5).

Table 5: Bacteria employed for the treatment of distillery wastewater.

S. No.	Name	Comments	Colour removal (%)	Reference
1.	*Xanthomonas fragariae*	All three strains needed glucose as carbon source and NH_4Cl as nitrogen source. The decolourisation efficiency of free cells was better than immobilized cells.	76	[14]
2.	*Bacillus megaterium*		76	
3.	*Bacillus cereus*		82	
4.	*Bacillus smithii*	Decolourisation occurred at 55° C in 20 days under anaerobic conditions in presence of pepton or yeast extract as supplemental nutrient. Strain could not use MWW as soul carbon source.	35.5	[151]
5.	*Lectobacillus hilgadii*	Immobilized cells of the hetero fermentative lactic acid bacterium decolourised 40% of the melanoidins solution within four days aerobically.	40	[151]
6.	*Acetobacter acetii*	The organism required sugar especially, glucose and fructose for decolourisation of MWWs.	76.4	[12]
7.	*Pseudomonas fluorescens*	This decolourisation was obtained with cellulose carrier coated with collagen. Reuse of decolourised cells reduced the decolourisation efficiency.	94	[52]
8.	*Pseudomonas putida*	The organism need glucose as a carbon source, to produce hydrogen peroxide which reduced the colour	60	[4]
9.	*Acinetobacter* sp.	All these organisms were isolated from an air bubble column. Reactor treating winery waste water after 6 months of operation. Most isolates from the colonized carriers belonged to species of the genus Bacillus.	Not checked in this study	[138]
10.	*Aeromonas sp.*			
11.	*Alcaligens faecalis*			
12.	*Bacillus sp.*			
13.	*Flavobacterium sp.*			
14.	*F. meningosepticum*			
15.	*Pseudomonas sp.*			
16	*P. paucimobilis*			
17.	*P. vescicularis*			
18.	*Sphingobacterium multivorum*			
19.	*Bacillus thuringiensis*	Addition of one percent glucose as a supplementary carbon source was necessary.	22	[153]
20.	*Bacillus brevis*		27.4	
21.	*Bacillus sp.*		27.4	
22.	*Pseudomonas*	The three strains were part of a consortium which decolourized aeruginosa the anaerobically digested spent wash in presence of basal salts and glucose.	67	[8]
23.	*Stenotrophomonas maltophila*			
24.	*Proteus mirabilis*			

Actinomycetes treatment of wastewater. Until 1992, there had been hardly any report on the decolourization of molasses melanoidin by actinomycetes. But Murata *et al.* [159] recovered a strain of *Streptomyces werraensis* TT14 after extensive screening of about 75 actinomycetes, which decolourized the model melanoidin pigment prepared from glucose and glycine. The strain TT14 showed the highest decolourizing activity 64% in optimum medium (2.0% starch, 1.0% yeast, 0.3% NaCl, and 0.3% $CaCO_3$) and 45% in a synthetic medium at pH 5.5 and 40°C for 4-days. They also tested the properties of decolourized melanoidin and reported that Cuchelating activity of decolourized melanoidin was decreased to about half of the intact melanoidin. The electro focussing patterns of melanoidin also differ from each other. A component of iso electric point (PI) 2.45, not existing in intact melanoidin was formed in microbial treated melanoidin. The melanoidin component of PI 2.5 was increased and that of PI 3.5 was reduced by the microbial treatment.

Yeast Treatment of distillery wastewater. Yeast, *Citeromyces* is most studied organism for treating MWW and high and stable removal efficiencies in both colour intensity and organic matter have been recorded [152]. Malandra *et al.* [144] yeast isolates which was able to reduce the COD of synthetic wastewater by 95% and 46% within 24 h under aerated and non-aerated conditions, respectively. Two flocculant strains of yeast, *Hansenula fabianii* and *Hansenula anomala* was used for the treatment of wastewater from beet molasses-spirits production and achieved 25.9% and 28.5% removal of TOC respectively from wastewater without dilution [153]. Dilution of wastewater was not favourable for practical treatment of wastewater due to the longer treatment time and higher energy cost. Color removal from MSW using terrestrial white-rot fungi was shown to be Mn-P dependent in *Phanerochaete chrysosporium* Dehorter and Blondeau [160] and laccase dependent in *Trametes versicolor* [161]. The process was sorbose oxidase and glucose oxidase-dependent in mitosporic fungi *Aspergillus fumigates* [136] and *A. oryzae* [162] and in the basidiomycete *Coriolus* sp. No. 20 [125]. It was demonstrated that MnP-independent decolourization of MSW by the marine-derived fungus NIOCC#312 which decolourized 60% of MSW when added at 50% concentration in seawater medium. There was a direct correlation between concentration of glucose oxidase and decolourization of MSW [34]. As previously discussed in this review, that decolourization was dependent on glucose oxidase levels in the culture medium like bacterial decolourization, it was suggested that H_2O_2 produced by glucose oxidase act as a bleaching agent. It was further demonstrated that marine fungi are capable of decolorizing MSW effectively in the presence of seawater of 15-34 ppt salinity [150].

C. Potential enzymetic treatment of distillery wastewater

Although the enzymatic system related with decolourization of melanoidins is yet to be completely understood, and it seems greatly connected with fungal ligninolytic mechanisms. The white-rot fungi have a complex enzymatic system which is extracellular and non-specific, and under nutrient-limiting conditions is capable of degrading lignolytic compounds, melanoidins, and polyaromatic compounds that cannot be degraded by other microorganisms [130]. A large number of enzymes from a variety of different plants and microorganisms have been reported to play an important role in an array of waste treatment applications. Several studies regarding degradation of melanoidins, humic acids and related compounds using basidiomycetes have also suggested a participation of at least one laccase enzyme in fungi belonging to *Trametes* (*Coriolus*). The role of enzymes other than laccase or peroxidases in the decolourization of melanoidins by *Trametes* (*Coriolus*) strain was reported during the 1980s. Several reports claimed that intracellular sugar-oxidase- type enzymes (sorbose-oxidase or glucose-oxidase) had melanoidin-decolourizing activities. It was suggested that melanoidins were decolourized by the active oxygen (O_2; H_2O_2) produced by the reaction with sugar oxidases [125]. Decolourization by microbial methods includes the enzymatic breakdown of melanoidin and flocculation by microbially secreted substances. Ohmomo *et al.* [163] used *C. versicolour* Ps4a, which decolourized molasses wastewater 80% in darkness under optimum conditions. Decolourization activity involved two types of intracellular enzymes, sugar-dependent and sugar-independent. One of these enzymes required no sugar and oxygen for appearance of the activity and could decolourize MWW up to 20% in darkness and 11–17% of synthetic melanoidins. Thus, the participation of these H_2O_2 producing enzymes as a part of the complex enzymatic system for melanoidin degradation by fungi should be taken into account while designing any treatment strategy. One of the more complete enzymatic studies regarding melanoidin decolourization was reported by Miyata *et al.* [164]. Colour removal of synthetic melanoidin by *C. hirsutus* involved the participation of peroxidases (MnP and MIP) and the extracellular H_2O_2 produced by glucose-oxidase, without disregard of a partial participation of fungal laccase. Mansur *et al.* [165] obtained a maximum decolourization of around 60% on day 8 after inoculating with fungus *Trametes* sp. I-62.

Here effluent was added at a final concentration of 20% (v/v) after 5 days of fungal growth, the time at which high levels of laccase activity were detected in the extracellular mycelium. The white-rot basidiomycete, *T. versicolour* is an active degrader of humic acids as well as of melanoidins. A melanoidin mineralizing 47 kDa extracellular proteins corresponding to the major mineralizing enzyme system from *T. versicolour* was isolated by Dehorter and Blondeau [160]. This Mn^{2+} dependent enzyme system required oxygen and was described to be as peroxidase. Uniform, small and spongy pellets of the fungus *T. versicolour* were used as inoculum for colour removal using different nutrients such as ammonium nitrate, manganese phosphate, magnesium sulphate and potassium phosphate and also sucrose as carbon source [130]. Maximum colour removal of 82% and 36% removal of $N–NH^{+4}$ was obtained on using low sucrose concentration and KH_2PO_4 as the only nutrient. Some studies have identified the lignin degradation related enzymes participating in the melanoidin decolourization. Intracellular H_2O_2 producing sugar oxidases have been isolated from *Coriolus* strains. Also, *C. hirsustus* have been reported to produce enzymes that catalyze melanoidin decolourization directly without additions of sugar and O_2. Miyata *et al*. [164] used *C. hirsutus* pellets to decolourize a melanoidin-containing medium. It was elucidated that extracellular H_2O_2 and two extracellular peroxidases, a manganese-independent peroxidase (MIP) and manganese peroxidase (MnP) were involved in decolourization activity. Lee *et al*. [166] investigated the dye-decolourizing peroxidase by cultivating *Geotrichum candidum* Dec1 using molasses as a carbon source. Components in the molasses medium stimulated the production of decolourizing peroxidase but inhibited the decolourizing activity of the purified enzyme. It was found that the inhibitory effect of molasses can be eliminated at dilution ratios of more than 25. Recently D'souza *et al*. [150] reported 100% decolourization of 10% spent wash by a marine fungal isolate whose laccase production increased several folds in the presence of phenolic and non-phenolic inducers. A combined treatment technique consisting of enzyme catalyzed in situ transformation of pollutants followed by aerobic biological oxidation was investigated by Sangave and Pandit [167] for the treatment of alcohol distillery spent wash. It was suggested that enzymatic pretreatment of the distillery effluent leads to in situ formation of the hydrolysis products, which have different physical properties and are easier to assimilate than the parent pollutant molecules by the microorganisms, leading to faster initial rates of aerobic oxidation even at lower biomass levels. In another study, Sangave and Pandit [168] used irradiation and ultrasound combined with the use of an enzyme as pretreatment technique for treatment of distillery wastewater. The combination of the ultrasound and enzyme yielded the best COD removal efficiencies as compared to the processes when they were used as stand-alone treatment techniques. Enzymatic decolourization of molasses medium has also been tried using *P. chrysosporium* [151]. Under stationary cultivation conditions, none of the strains could decolourize molasses nor produce enzymes lignin peroxidase, manganese peroxidase and laccase. All of them could produce lignin peroxidase and manganese peroxidase when cultivated in flat bottom glass bottles under stationary cultivation conditions.

D. Microbial consortium treatment

During last two decades, several attempts have been made to investigate the possibility of using cell immobilization in the technology of aerobic wastewater treatment [169-170]. Early experiments were restricted to the use of selected pure cultures immobilized on solid supports for the degradation of specific toxic compounds [171-172]. Later, immobilized consortia of two or more selected strains were employed [173-174] but of late activated sludge has been immobilized on different carriers and used for wastewater treatment [175]. Jet loop reactors (JLR), the efficiency of which has already been shown in both chemical and biological processes have also been evaluated for aerobic treatment of winery wastewater. A JLR of 15 dm3 working volume was used for the aerobic treatment of winery wastewater [176]. COD removal efficiency higher than 90% was achieved with an organic load of the final effluents that ranged between 0.11 and 0.3 kg COD m^3. Most isolates belong to the genus *Pseudomonas* and the yeast *Saccharomyces cerevisiae*. Later, Eusibio *et al*. [30] reported the operation of a JLR for more than one year treating winery wastewater collected in different seasons and achieved an average COD removal efficiency of 80%. JLR have higher oxygen transfer rates at lower energy costs. They also observed *Bacillus* apart from *Pseudomonas* and the yeast *Saccharomyces cerevisiae*. Adikane *et al*. [177] studied decolourization of molasses spent wash in absence of any additional carbon or nitrogen source using soil as inoculum. A decolourization of 69% was obtained using 10% (w/v) soil and 12.5% (v/v) MSW after 7 days incubation.

E. Immobilized Bioreactors

Cells of *Phanerochaete chrysosporium* immobilized in calcium–alginate beads resulted in a much more rapid decolourization of MSW than did free cells [154]. Maximum color reduction occurred between 0 and 2 days.

However, the color elimination was reduced from 85% with free cells to 59% with immobilized cells after 10 days. The immobilized *Coriolus versicolor* on nylon cubes in a packed-bed bioreactor eliminated the COD of the pretreated effluent by a further 50.3%, reaching a total reduction of 77% [178]. Only 4% color was eliminated, and this was due primarily to absorption onto the fungal mycelia rather than enzymatic oxidation. It is possible to bioremediate such spent waste using a multistage treatment process with an initial treatment with *Geotrichum candidum*. In one study it was shown that immobilized *Flavodon flavus* in $1cm^3$ of polyurethane foam could be used effectively for three consecutive cycles of decolourization of fresh 10% MSW [137]. The fungus also removed about 98% of the toxicity of the MSW using an estuarine fish, *Oreochromis mossambicus*. Benzopyrene was present in the MSW and appeared to be one of the causes of toxicity. The total PAH concentration in the MSW was reduced by 68% after 5 days. This is a first report of decolourization of MSW along with simultaneous detoxifi cation and reduction in the PAH content of the MSW. Four fungi-*Penicillium* sp., *Penicillium decumbens*, *Penicillium lignorum*, and *Aspergillus niger* produced maximum decolourization of beet molasses alcoholic fermentation wastewater on the fourth day of treatment in a study by Jimenez *et al*. [133]. *P. decumbens* produced a maximum decolourization of 40%. Four fungi reduced an average of 70% of the phenolic content of the wastewater. *Penicillium* sp. and *P. decumbens* removed 52.1% and 50.7% of the COD, respectively, on the fifth day of fermentation. Anaerobic digestion of previously fermented *P. decumbens* beet molasses was carried out in suspended cell bioreactors. This treatment removed an average of 93% COD with a methane yield coefficient of 305ml of methane at STP per gram of COD removal. This combined aerobic–anaerobic treatment removed 96.5% of the COD and reduced the HRT to eliminate COD. The VFA/alkalinity ratio has been used as a measure of process stability [179]. The VFA/alkalinity ratio was 1.4 and 0.6 for untreated and fermented molasses, respectively, which suggests the removal of volatile fatty acids from the respective wastewater by anaerobic biomasses, with the system reaching equilibrium. Anaerobic digestion of untreated and treated molasses followed the first-order kinetics for biomass loading rates in the range 0 to 0.55 and 0 to 0.75 g of COD per gram of volatile suspended solids (VSS) per day, respectively. Strong brewery waste has been trickled through a vertical curtain of two 3 mm layers of reticulated polyurethane foam bonded to a reinforcing nylon cloth core [180]. *Geotrichum fragrans* adhered tenaciously to the curtain and acted as a matrix for other yeasts or bacteria. A thick lawn was produced by this process in which a lack of nutrition to cells forced impairment of the curtain. This curtain configuration acts as a self-reproducing cell immobilization on a solid support. Ninety four percent of the COD was reduced (from 55 000 mg/l to 3300 mg/l) by passing through 6 m of curtain. A 1 m^2 curtain with a 4-m fall can treat 15 l/d.

F. Effect of environmental factors on treatment process

Several environmental factors, such as sources of carbon, nitrogen, phosphorus, pH, temperature, aeration and inoculums dose play vital role in microbial degradation process of industrial wastes as the activity of enzymes is greatly influenced by these environmental factors. The role of various environmental factors in degradation of melanoidins to achieve the maximum degradation and decolourization by different microbes [9, 13, 158]. However, Hayase *et al*. [181] while studying the degradation of melanoidins by hydrogen peroxide at different pH (3.0–13.0) found that melanoidin decolourization in alkaline pH proceeds more rapidly than in acidic and neutral pH and it reached 94% at pH 10. Mohana *et al*. [9] have reported the highest decolourization (67%) at pH 7.0 as the solubility of melanoidins depends on pH i.e. it is less soluble in acidic pH than in alkaline pH and pH more or less than pH 7.0 led to decrease in decolourization activity as well as the growth of microbes. The best decolourization (80%) and highest COD removal (75%) at on initial pH of 5 [130]. They have also observed that increased in temperature from 20 to 37°C was accompanied with increased in decolourization from 35% to 67% and further increase in temperature above 40°C adversely affected the decolourization ability of microbes as melanoidin decolourizing ability of microbial cells was lost on exposure at high temperature (>40°C) for long time. Temperature influences growth, metabolism, and nutrition, enzymes, biomass and cell permeability. The optimum growth and highest color removal occurred at 30°C for the strain D90 [135], 35°C for *Coriolus versicolor*, and 40°C for *Phanerochaete chrysosporium* [134]. The availability of carbon source is a requirement for the growth of fungi and melanoidin removal. Ohmomo *et al*. [128] have studied the effect of various carbon and nitrogen sources on the MDA of *Aspergillus fumigatus* G-2-6 and reported that glucose was the best carbon source allowing the maximum degradation of melanoidins and further increase in glucose concentration (>1%) resulted only increase in mycelial biomass but no further increase in decolourization yield. Nitrogen is a component of cell wall polymers and is important for protein synthesis. A low-nitrogen medium favours the highest decolourization of distillery effluent by *Flavodon flavus* [34].

Among the various nitrogen sources, yeast extract, peptone, beef extract and tryptone brought down the level of decolourization whereas inorganic nitrogen sources such as sodium nitrate and ammonium nitrate reduced the decolourization values. It has also been observed that decolourization of melanoidins increases with increase in inoculums size and maximum decolourization was achieved at 15% (v/v) inoculums size (approx. 11×10^6 CFU/ml) and further increase in inoculum size did not improve the decolourization of melanoidins [9]. Dahiya *et al*. [54] demonstrated a 5% (w/v) dry weight of mycelial suspension of *Phanerochaete chrysosporium* JAG-40 to be optimal for maximum decolourization in melanoidin medium supplemented with glucose and peptone. Colour elimination and COD reduction occurred with different inoculums mycelial concentrations in batch cultures of *Trametes versicolor* [130].

V. CONCLUSION

Coloured effluent treatment and decolourization arduous task. This review indicates that, a wide range of biological as well as physic-chemical treatments have been investigated over the years for the treatment of distillery effluent. These medium to high strength organic and inorganic waste effluent are produced in vast amount of acidic and recalcitrant coloured compound, all of which have been successfully removed by a number of biological and physic-chemical and post treatment technologies.

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