



Removal of melanoidin present in distillery effluent as a major colorant: A Review

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Abstract: Effluent originating from distilleries contain large amount of dark brown coloured wastewater called molasses spent wash (MSW). This MSW is the unwanted residual liquid waste to dispose because of low pH, high temperature, dark brown colour, high ash content, unpleasant odour and high percentage of organic and inorganic matter. Dark brown colour of MSW is due to the presence of melanoidin pigment. It reduces sunlight penetration in rivers and lakes which in turn decrease both photosynthetic activity and dissolved oxygen concentration affecting aquatic life. So the disposal of this effluent is one of the critical environmental issues. A number of treatment processes have been employed for the distillery waste management. This review paper present an overview of the pollution problems caused by melanoidin and the technologies employed globally for its removal.

Key words: Colour, Effluent, Melanoidin, Waste management, Degradation
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Introduction

The wastewater released from distilleries is known as spent wash, which is highly acidic in nature. In the year 1999, there were 285 distilleries in India producing 2.7×10^9 l of alcohol and generating 4×10^{10} l of wastewater each year (Joshi, 1999). This number has gone up to 319, producing 3.25×10^9 l of alcohol and generating 40.4×10^{10} l of wastewater annually (Uppal, 2004). Because of using large quantities of water in distillery industries it is essential to treat and reuse their waste water. In the most of time the discharge standards applied for distilleries are often too stringent and below the level that can be achieved with appropriate biological treatment technologies (Pant and Adholeya, 2007a,b). The production and characteristics of spent wash is highly variable and dependent on feed stocks and various aspects of the ethanol production process (Durate *et al.*, 1997). The molasses spent wash (MSW) is a potential water pollutant in two ways. First, the highly coloured nature of MSW can block out sun light from rivers and streams thus reducing oxygenation of the water by photosynthesis and hence becomes detrimental to aquatic life. Secondly, it has a high pollution load which would result in eutrophication of contaminated water sources (FitzGibbon *et al.*, 1998). The first reason is due to the presence of water soluble recalcitrant colouring compound called melanoidin (Evershed *et al.*, 1997). Melanoidin are dark brown to black coloured natural condensation product of sugar and amino acids produced by nonenzymatic browning reactions called maillard reactions (Plavsic *et al.*, 2006). Ohmomo *et al.* (1988a) concluded that microbial decolourization of melanoidin is due to two decomposition mechanisms; in the first the smaller molecular weight melanoidin are attacked and in the second the larger molecular weight melanoidin are attacked. Satyawali and Balakrishnan

(2008a,b) have investigated that the degradation of low molecular weight compound occurred in the membrane bioreactor (MBR) while the higher molecular weight compounds comprising the colour imparting melanoidin remained unaffected.

Physico-chemical characteristics: The distillery spent wash is hot, highly coloured and acidic with strong and objectionable odour that presents significant disposal or treatment problem. Physico-chemical characteristics are given in Table 1.

Effect of distillery effluent: The wastewater released from distilleries and fermentation industries are the major source of soil and aquatic pollution due to presence of water soluble recalcitrant colouring compounds called melanoidin (Evershed *et al.*, 1997). Melanoidin are high molecular weight amino-carbonyl compounds. Due to their structural complexity, dark colour and offensive order, these pose serious threat to soil and aquatic eco system that release of melanoidin cause increased load of recalcitrant organic material to natural water bodies. This then causes the problem, like reduction of sun light penetration, decreased photosynthetic activity and dissolved oxygen concentration whereas on land, it causes reduction in soil alkalinity and inhibition of seed germination. Further due to the possibility of complexation reaction of introduced melanoidin with metal ions, they could influence the biogeochemical cycle of many constituents in natural water (Chandra *et al.*, 2008), which are highly resistant to microbial attack. Hence the wastewater requires pre treatment before its safe disposal in to the environment (Mohana *et al.*, 2007; Kumar and Chandra, 2006).

Conventional biological processes such as activated sludge treatment process are insufficient to treat these melanoidin containing wastewater released from distilleries. Degradation and

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decolourization of these wastewater by chemical methods (Chandra and Singh, 1999), flocculation treatment and physicochemical treatment such as ozonation (Kim *et al.*, 1985) and activated carbon adsorption have been accomplished, but these methods are not economically feasible on large scale due to cost limitation where as biological decolourization by using fungi such as *Coriolus*, *Aspergillus*, *Phanerochaete* and certain bacterial species such as *Bacillus*, *Alkaligenes* and *Lactobacillus* (Kumar and Chandra, 2006; Kumar *et al.*, 1997; Ohmomo *et al.*, 1987, 1985; Aoshima *et al.*, 1985) have been successfully achieved and thus can be applied as a bioremediation technique.

Biological treatment methods :

Bacterial treatment: Pure bacterial culture for microbial treatment has been reported frequently in past and recent years. Under aerobic condition *Bacillus* sp. has been decolourize molasses wastewater upto 35.5% with in 20 days at 55°C (Nakajima *et al.*, 1999). A detailed list of bacteria tried by different researchers for decolourization of distillery effluent is given in Table 2. Kumar and Viswanathan (1991) isolated bacterial strains from sewage and acclimatized on increasing concentrations of distillery waste. These strains were able to reduce COD by 80% in 4-5 days without any aeration. The major products left after treatment were biomass, carbon dioxide and volatile acids. Dahiya *et al.* (2001a) isolated *Pseudomonas fluorescens* from reactor liquid and found that these bacterial strains are capable of decolourizing melanoidin wastewater upto 76% under nonsterile condition and upto 90% in sterile condition. The difference in decolourization might be due to the fact that melanoidin stability varies with pH and temperature. At higher temperature during sterilization melanoidin-pigments decompose to low molecular weight compounds (Ohmomo *et al.*, 1988b). The pH of distillery spent wash increases from 4.5 to 8.5 during the anaerobic treatment process and finally effluent is called post methanated distillery effluent (PMDE). Many workers have isolated several aerobic and anaerobic strains and studied the degradation and decolourization of PMDE in terms of decrease in absorbance at 475 nm, bacterial growth at 620 nm, increase in biomass and reduction in colour intensity have been studied by many workers with the help of isolated aerobic and anaerobic strains (Mohana *et al.*, 2007; Adikane *et al.*, 2006; Kumar and Chandra, 2006; Sirianuntapiboon *et al.*, 2004; Benito *et al.*, 1997). Ohmomo *et al.* (1988a) used calcium alginate immobilized cells of *Lactobacillus hilgardii* to decolourize melanoidin solution which resulted in 40% decolourization. It requires a small amount of oxygen continuously with limited aeration for the decolourization. Some researchers carried out melanoidin decolourization by using immobilized whole cells. Decolourization of molasses wastewater by immobilized cells of *Pseudomonas fluorescence* on porous cellular carrier was attempted achieving 76% decolourization in 24 hr at 30°C. Jain *et al.* (2002) isolated three bacterial strains from the activated sludge of a distillery effluent identified as a *B. megaterium*, *B. cereus* and *B. fragariae* which were found to remove colour and COD from the distillery effluent in the range of 38-58 and 55-68%, respectively. An Acetogenic strain was isolated by Sirianuntapiboon *et al.* (2004)

from vegetables and juice samples which decolorizes molasses pigment medium and anaerobically treated distillery effluent to 73-76% with in 5 days when supplemented with glucose and nitrogen sources. Patel *et al.* (2001) have reported 96, 81 and 26% decolourization of distillery effluent through bioflocculation by *Oscillatoria sp.*, *Lyngbya sp.* and *Synechocystis sp.* respectively. Ghosh *et al.* (2004) have also isolated some bacterial strains capable of degrading recalcitrant compounds from anaerobically digested spent wash isolated from soil of effluent discharge site. These were *Pseudomonas*, *Enterobacter*, *Stenotrophomonas*, *Klebsilla* and *Acinetobacter*, all of which carried out degradation of PMDE and maximum 44% COD reduction either singly or collectively. Cibis *et al.* (2002) achieved biodegradation of potato slops (distillation residue) by a mixed population of bacteria under thermophilic conditions up to 60°C. A COD removal of 77% was achieved under non-optimal conditions. Marine cyanobacteria such as *Oscillatoria boryna* have also been reported to degrade melanoidin due to production of H₂O₂, hydroxyl, perhydroxyl and active oxygen radicals, resulting in the decolourization of the effluent (Kalavathi *et al.*, 2001). Sirianuntapiboon *et al.* (2004) used an acetogenic bacterium to obtain a decolourization yield of 76.4% under optimal nutrient conditions. However, this value was only 7.3%, by using anaerobic pond. Also, it required sugar, especially glucose and fructose for decolourization of MSW. The decolourization activity might be due to a sugar oxidase.

Fungal treatment: In recent years, several basidiomycetes and ascomycetes type fungi have been used in the decolourization of natural and synthetic melanoidin in connection with colour reduction of wastewaters from distilleries. The fungus have capability to purify the effluent by consumption of organic substances, thus, reducing its COD and BOD, and at the same time to obtain some valuable product, such as fungal biomass for protein-rich animal feed or some specific fungal metabolite. In comparison to bacteria filamentous

Table - 1: The Physico-chemical characteristics of distillery effluent (Mean ± SE of mean)

S.No.	Parameters	Value	BIS
1	T°C	28.5±.23	40
2	Colour	Reddish	None
3	Total Solids (mg l ⁻¹)	4285±.005	2100
4	Total Dissolved Solids (mg l ⁻¹)	3980±.15	2100
5	Total Suspended Solids (mg l ⁻¹)	255±.005	100
6	pH	6.4±.003	5.5-9
7	Total Alkalinity (mg l ⁻¹)	1437.5±.29	-
8	Total Hardness (mg l ⁻¹)	565±.005	300
9	Calcium Hardness (mg l ⁻¹)	455±.005	-
10	Calcium (mg l ⁻¹)	182.2±.11	169
11	Chloride (mg l ⁻¹)	860.87±.40	600
12	DO (mg l ⁻¹)	Nil	4 - 6
13	BOD (mg l ⁻¹)	544.5±.50	30
14	COD (mg l ⁻¹)	2433.38±.17	250

DO = Dissolved oxygen, BOD = Biochemical oxygen demand, COD = Chemical oxygen demand, BIS = Beuro of Indian Standard
Source: Pandey *et al.*, 2007

Table - 2: Bacteria employed for the decolourization of distillery effluent

Name	Comments	Colour removal (%)	Reference
<i>Xanthomonas fragariae</i>	All the three strains needed glucose as carbon source and NH ₄ Cl as nitrogen source. The decolourization efficiency of free cells was better than immobilized cells.	76	Jain <i>et al.</i> , 2002
<i>Bacillus smithii</i>	Decolourization occurred at 55°C in 20 days under anaerobic conditions in presence of peptone of yeast extract as supplemental nutrient. Strain could not use MWW as sole carbon source	35.5	Kambe <i>et al.</i> , 1999
<i>Lactobacillus hilgardii</i>	Immobilized cells of the hetero fermentative lactic acid bacterium decolourized 40% of the melanoidin solution within 4 days aerobically	40	Ohmomo <i>et al.</i> , 1988a
<i>Acetobacter acetii</i>	The organism required sugar especially, glucose and fructose for decolourization of MSWs	76.4	Sirianuntapiboon <i>et al.</i> , 2004
<i>Pseudomonas Fluorescens</i>	The decolourization was obtained with cellulose carrier coated with collagen. Reuse of decolourized cells reduced the decolourization efficiency	94	Dahiya <i>et al.</i> , 2001a
<i>Bacillus thuringiensis</i>	Addition of 1% glucose as a supplementary carbon source was necessary	22	Kumar and Chandra, 2006
<i>Pseudomonas aeruginosa</i>	The three strains were part of a consortium which decolourized the anaerobically digested soeibt wash in presence of basal salts and glucose	67	Mohana <i>et al.</i> , 2007

fungi have lower sensitivity to variations in temperature, pH, nutrients and aeration and have lower nucleic acid content in the biomass (Knapp *et al.*, 2001). One of the most studied fungus having ability to degrade and decolourize distillery effluent is *Aspergillus* sps. such as *Aspergillus fumigatus* G-2-6, *A. niger*, *A. niveus*, *A. fumigatus* Ub²60 brought about an average of 69-75% decolourization along with 70-90% COD reduction (Ohmomo *et al.*, 1987; Miranda *et al.*, 1996; Jimnez *et al.*, 2003). Several fungi have been investigated for their ability to decolourize melanoidin from MSW in Table 3. *Coriolus* sp. no. 20, in class basidiomycetes was the first strain for the application of its ability to remove melanoidin from MSW (Watanabe *et al.*, 1982). This isolate did not show any decolourization activity when molasses pigment was used as carbon source but it showed the activity when sorbose or glucose was added. In 1985, Ohmomo *et al.* used *Coriolus versicolour* Ps4a for MSW decolourization and obtained 80% decolourization in darkness under optimum conditions. Later, Ohmomo *et al.* (1988b) used autoclaved mycelium of *Aspergillus oryzae* Y-2-32 that adsorbed lower weight fractions of melanoidin and degree of adsorption was influenced by the kind of sugars used for cultivation. Jimnez *et al.* (2003) reported the treatment of distillery spent wash with ascomycetes group of fungi such as *Penicillium* sps. for example *P. decumbens*, *P. lignorum* resulted in about 50% reduction in colour and COD and 70% phenol removal. Sirianuntapiboon *et al.* (1995) studied that *Rhizoctonia* sp. D-90 decolourized molasses melanoidin medium and a synthetic melanoidin medium by 87.5 and 84.5%

respectively, under experimental growth conditions. Electron microscopy revealed that the mycelia absorbed melanoidin pigment, which was in the form of electron dense material in the cytoplasm. However, melanoidin could be eluted from the mycelia by washing in a solution of NaOH and the relative amount of melanoidin eluted from the mycelia increased with increase in the concentration of NaOH. Kida *et al.* (1995) has investigated that *Aspergillus awamori* var. *kawachi* used for production of single cell protein from Japanese distillery (Shochu) wastewater after aerobic cultivation. The supernatant after cultivation could be anaerobically treated, at a high TOC loading rate, by the addition of Ni²⁺ and Co²⁺. Also, NH₄⁺ accumulated in the anaerobically treated wastewater, was efficiently removed by utilization of residual volatile fatty acids (VFA) as electron donors during biological denitrification and nitrification and the residual organic matter could be removed simultaneously. Miranda *et al.* (1996) studied that Colour elimination from MSW by using *Aspergillus niger* and they found that under optimal nutrient concentration 83% of the total colour removed was eliminated biologically and 17% by adsorption on the mycelium. Benito *et al.* (1997) have investigated that anaerobically treated distillery effluent when supplemented with sucrose and inorganic N source has capability to decolourize by the *Trametes versicolor*. It was found that reduction in COD was 75% and decolourization was 80%. Under nutrient limiting conditions, fungal cells generally cannot remain active during a long-term cultivation. Therefore, the continuous-culture method is not practical and the semi-batch or repeated-batch method can be

an alternative for long-term cultivation. The immobilization of the fungus on a solid support is an appropriate means for controlling the thickness of the biofilm. The immobilization of the fungus offers advantages such as short retention time, easy recovery of the cells and increased activity. Furthermore, in the presence of the foam matrix, pellet size is restricted by the size and the physical properties of the foam (Kim and Shoda, 1999). Recently, Pant and Adholeya (2007a,b) isolated three fungal strains and identified them by molecular methods as *Penicillium pinophilum* TERI DB1, *Alternaria gaisen* TERI DB6 and *Pleurotus florida* EM 1303. These cultures were found to produce lignolytic enzymes and decolourize the effluent upto 50, 47 and 86% respectively. Miyata et al. (2000) suggested an inhibitory effect of organic nitrogen on melanoidin decolourization by fungus *Coriolus hirsutus*. At the same time glucose was also required for enhancing decolourization as the peroxidases require H₂O₂, which is generated by glucose oxidation, to decolourize melanoidin. In another study it was reported that presence of additional nitrogen could not inhibit activity of fungus *C. versicolour* sp. no. 20 considerably, as significant decolourization and COD reduction occurred even in the absence of it (Chopra et al., 2004).

Raghukumar and Rivonkar (2001) have reported that colour removal from distillery effluent using a marine fungus, *Flavodon avus*. This fungus was more effective in decolourizing raw MSW than was a molasses wastewater collected either after anaerobic treatment or after aerobic treatment. The oxygen demand of the fungus was quite high. *P. chrysosporium* JAG-40 decolourized synthetic and natural melanoidin present in spent wash up to 80% (Dahiya et al., 2001b). Sirianuntapiboon et al. (1995) reported that the decolourization of melanoidin pigment by *Rhizoctonia* sp. D-90 by adsorption mechanism. The pigment was accumulated in the cytoplasm and around the cell membrane as melanoidin complex, which was gradually decolourized by intracellular enzyme. The larger molecular weight fractions of melanoidin were decolourized rapidly, while the small molecular weight fractions remained in solution and were metabolized slowly. Also, the decolourization was less in sterilized spent wash than in non-sterile solution. *Aspergillus niveus*, a litter degrading fungus was used by Angayarkanni et al. (2003) for the treatment of distillery effluent using paddy straw, sugarcane bagasse, molasses and sucrose as carbon source for growth of fungus in the effluent. Sugarcane bagasse at 1% (w/v) concentration resulted in maximum removal of colour (37%) and COD (91.68%). The decrease in colour removal in this study might be due to the fact that the effluent taken for study was alkaline (pH 9.0) and the melanoidin responsible for colour were more soluble in the alkaline pH. In the acidic pH, the melanoidin might be precipitated and removed easily. Shayegan et al. (2005) used an *Aspergillus* species isolated from the soil for decolourization of anaerobically digested (UASB) and aerobically treated distillery wastewater. With diluted wastewater at optimum values of supplemented materials 75% decolourization was achieved which reduced to 40% on using undiluted wastewater. It was suggested that decolourization by fungi takes place due to the destruction of coloured molecules and partially

because of sorption phenomena. A longer aeration period causes the adsorbed colour molecules to be released as a result of endogenous respiration and cell death, hence reducing decolourization efficiency. Yeast *Citeromyces* was used for treating MWW (melanoidin waste water) and high and stable removal efficiencies in both colour intensity and organic matter were obtained. However, the semi-pilot and pilot-scale experiments are to be tested for checking the stability of *Citeromyces* sp. (Sirianuntapiboon et al., 2003).

Mixed 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 (Fedrici, 1993; Sumino et al., 1985). Early experiments were restricted to the use of selected pure cultures immobilized on solid supports for the degradation of specific toxic compounds (Anselmo et al., 1985; Livernoche et al., 1983). Later, immobilized consortia of two or more selected strains were employed (Kowalska et al., 1998; Zache and Rehm, 1989) but of late activated sludge has been immobilized on different carriers and used for wastewater treatment (Shah et al., 1998). Adikane et al. (2006) 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.

Phytoremediation approach: Phytoremediation of effluents is an emerging low cost technique for the removal of toxicants including metals from industrial effluent and is still in an experimental stage (Mohana et al., 2009). Kumar and Chandra (2004) successfully treated distillery effluent in a two stage process involving transformation of recalcitrant colouring components of the effluent by a bacterium *Bacillus thuringiensis* 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 *B. thuringiensis* and *Typha angustata* by Chandra et al. (2008) which resulted in 98-99% BOD, COD and colour reduction after 7 days. Recently, macrophyte *Potamogeton pectinatus* was used for bioaccumulating heavy metals from distillery effluent (Singh et al., 2005). Trivedy and Nakate (2000) employed *Typha latifolia* for distillery effluent treatment in a constructed wetland and found that the system resulted in 78 and 47% reduction in COD and BOD respectively in a period of 10 days. Increasing concentration of the effluent greatly reduced the biomass of the plant with maximum accumulation of Fe being recorded in plants growing in 100% effluent. Valderrama et al. (2002) reported 52% colour removal from distillery effluent when using a combined treatment with *Lemna minuscula* and *Chlorella vulgaris*. The micro algal treatment removed nutrients and organic matter from wastewater and produced oxygen for other organisms. The macrophyte removed organic matter and eliminated the microalgae from treated wastewater. However, despite the potential of aquatic macrophytes in cleaning wastewaters the use of these plants in designing a low cost treatment system is still at experimental stage and is considered to be a potentially important area of environmental management.

Table - 3: Fungi employed for the decolourization of Distillery effluent

Name	Comments	Colour removal (%)	References
<i>Phanerochaete chrysosporium</i>	Free cells as well as calcium alginate immobilized cells decolourized the distillery effluent	85 (free) 59 (immobilized)	Fahy <i>et al.</i> , 1997
<i>Phanerochaete chrysosporium</i>	Both the fungi required a readily available carbon source for melanoidin decolourization while nitrogen source has no effect. Maximum decolourization was observed in 6.2% spent was.	53.5	Kumar <i>et al.</i> , 1998
<i>Trametes versicolour</i>	Anaerobically treated distillery effluent supplemented with sucrose and inorganic nitrogen sources was decolourized by the culture in shake flask studies	80	Benito <i>et al.</i> , 1997
<i>Penicillium sp.</i>	All fungi produced decolourization from first day of incubation, with maximum being show by <i>P.decumbens</i> at fourth day with a reduction of 70% of the phenolic content of the wastewater	30	Jimnez <i>et al.</i> , 2003
<i>Aspergillus niger UM2</i>	Decolourization was more by immobilized fungus and it was able to decolourize up to 50% of initial effluents concentrations.	80	Patil <i>et al.</i> , 2003
<i>Corilus hirsutus</i>	Synthetic as well as wastewater melanoidin was decolourized by the fungus in a median containing glucose and peptone.	80	Miyata <i>et al.</i> , 1998 and Miyata <i>et al.</i> , 2000
<i>Flavodon flavus</i>	Distillery effluents are decolourized using these marine basidiomycetes in presence of 5% glucose.	80	Raghukumar and Rivonkar, 2001; Raghukumar <i>et al.</i> , 2004
<i>Coriolus versicolour</i>	The cultures were incubated along with cotton stalks in vinasses media in static condition. No synthetic carbon and nitrogen sources were used	63	Kahraman and Yesilada, 2003
<i>Mycelia sterilia</i>	Organism requires glucose for the decolourizing activity	93	Sirianuntapiboon <i>et al.</i> , 1988
<i>Aspergillus niveus</i>	The fungus could use sugar bagasse as carbon source and require other nutrients for decolourization	56	Angayarkanni <i>et al.</i> , 2003
<i>Coriolus sp. no. 20</i>	First strain for the application of its ability to remove melanoidin from MWW, showed decolourization activity in 0.5% melanoidin when sorbose or glucose was added as a carbon source.	80	Watanabe <i>et al.</i> , 1982
<i>Aspergillus - UB2</i>	This was with diluted wastewater with optimum values of supplemented materials.	75	Shayegan <i>et al.</i> , 2005
<i>Phanerochaete chrysosporium</i> NCIM 1073 NCIM 1106 NCIM 1197	Molasses medium decolourization was checked in stationary and submerged cultivations conditions.	08276	Thakkar <i>et al.</i> , 2006
<i>Pleurotus florida</i> Eger EM1303	Hydroponically treated distillery effluent was subjected for treatment by fungus	86.3	Pant and Adholeya, 2009

Cyanobacterial treatment: Cyanobacteria are considered ideal for the treatment of distillery effluent as they, apart from degrading the polymers also oxygenated water bodies, thus reduce the BOD and COD levels (Mohana *et al.*, 2009). Kalavathi *et al.* (2001) explored the possibility of using a marine cyanobacterium for

decolourization of distillery spent wash and its ability to use melanoidin as a carbon and nitrogen source. A marine filamentous, non heterocystous form *Oscillatoria boryana* BDU92181 used the recalcitrant biopolymer melanoidin as nitrogen and carbon source leading to decolourization. First the microalgal treatment led to removal

of organic matter and further treatment with macrophytes removed other organic matter, colour and precipitated the microalgae.

Physicochemical treatment methods: After a multistage biological treatment of distillery spent wash, most of the organic load is removed. However, the brown colour does not disappear and may even increase due to repolymerization of the coloured components, melanoidin (Pena *et al.*, 2003). Conventional anaerobic and aerobic treatment can accomplish degradation of the melanoidin up to only about 6-7%. Therefore, it is necessary to study about additional treatments required to decolourize distillery effluent (Pena *et al.*, 2003). Majority of these methods remove colour by either concentrating the colour into sludge or by partial or complete breakdown of the colour molecules.

Adsorption: Among the physicochemical treatment methods, adsorption on activated carbon (AC) is widely employed for removal of colour and specific organic pollutants. Activated carbon is a well known adsorbent due to its extended surface area, microporous structure, high adsorption capacity and high degree of surface reactivity. Previous studies on decolourization of distillery spent wash include adsorption on commercial as well as indigenously prepared activated carbons (Satyawali and Balakrishnan 2008a,b). Bernardo *et al.* (1997) investigated decolourization of synthetic melanoidin using commercially available activated carbon as well as activated carbon produced by sugarcane bagasse. Chandra and Pandey (2000) observed that significant decolourization was achieved when used packed bed on anaerobically treated spent wash using commercial activated charcoal with a surface area of 1400 m² g⁻¹. Almost complete decolourization (> 99%) was obtained with 70% of the diluted sample.

Coagulation and flocculation: Coagulation is the destabilization of colloids by neutralizing the forces that keep them apart. Cationic coagulants provide positive electric charges to reduce the negative charge (zeta potential) of the colloids. As a result, the particles collide to form larger particles (flocs). Flocculation is the action of polymers to form bridges between the flocs, and bind the particles into large agglomerates or clumps. Bridging occurs when segments of the polymer chain adsorb on different particles and help particles aggregate. Generally coagulation seems to be an expensive step taking into account expenses of chemicals and sludge disposal. Thus, there is a need for development of low cost alternatives for post biomethanated effluent. Migo *et al.* (1993) used a commercial inorganic flocculant, a polymer of ferric hydroxysulphate for the treatment of molasses wastewater. The treatment resulted in around 87% decolourization for biodigested effluent. These findings have been in disagreement with those of Inanc *et al.* (1999) who reported that coagulation with alum and iron salts was not effective for colour removal. They explored lime and ozone treatment with anaerobically digested effluent. The optimum dosages of lime was found to be 10 g l⁻¹ resulting in 82.5% COD removal and 67.6% reduction in colour in a 30 min period. Later FeCl₃ and AlCl₃ were tested for decolourization of biodigested effluent and showed similar removal efficiencies, about 93% reduction in colour and 76% reduction in total organic carbon (Sowmeyan and Swaminathan, 2008).

Oxidation processes: Ozone is a powerful oxidant for wastewater treatment. Once dissolved in water, ozone reacts with a great number of organic compounds in two different ways: by direct oxidation as molecular ozone or by indirect reaction through formation of secondary oxidants like free radical species, in particular the hydroxyl radicals. Both ozone and hydroxyl radicals are strong oxidants and are capable of oxidizing a number of compounds (Bes-Pia *et al.*, 2003). Ozone destroys hazardous organic contaminants and that have been applied for the treatment of dyes, phenolics, pesticides, etc. (Pena *et al.*, 2003). The Fenton's oxidation technology is based on the production of hydroxyl radicals •OH, which have an extremely high oxidation potential. Fenton's reagent, which involves homogeneous reaction and is environmentally acceptable, is a mixture of hydrogen peroxide and iron salts (Fe²⁺ or Fe³⁺) which produces hydroxyl radicals which ultimately leads to decolourization of the effluent (Pala and Erden, 2005).

It has been observed that the use of an individual process alone may not treat the wastewater completely. A combination of these processes is necessary to achieve the desirable norms.

In general a biological treatment employing fungi and bacteria have been investigated essentially for decolourize the distillery spent wash. The microbial decolourization is an environment- friendly and cost competitive alternative to chemical decomposition process. Optimum microbial activities and optimum results are found when effluent is supplemented with additional nutrients as well as diluting the effluent. So it is felt that the ideal cost effective and commercial treatment scheme should comprise of physico-chemical treatment followed by biological treatment.

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