CHAPTER II

BIBLIOGRAPHY

2.1 Current production of sugarcane molasses in Thailand

Total Thai sugarcane production averaged 50–60 million tons a year from planted area of 1 million hectares (Office of Agriculture Economics [OAE], 2006). From this harvest, approximately 2.5–3 million tons of molasses is produced, 60-70% of which is locally utilized in many industries including food, feed and distillery, and the rest are supplied to export market (OAE, 2004). The increase in production of molasses in Thailand crops from 1988 and 2007 is shown in Table 1.

Table 1 Molasses production in Thailand for the crops years 1988/89-2006/07 (Office of the Cane and Sugar board [OCSB], 2007).

Crops Year	Molasses Production (million tons)				
	Northern	Central	Eastern	North Eastern	Total
1988/ 1989	0.324	0.981	0.204	0.242	1.742
1989/ 1990	0.288	0.645	0.208	0.278	1.719
1990/ 1991	0.480	1.091	0.197	0.400	2.168
1991/ 1992	0.555	1.169	0.208	0.470	2.402
1992/ 1993	0.411	0.743	0.151	0.318	1.623
1993/ 1994	0.455	0.870	0.157	0.436	1.918
1994/ 1995	0.618	1.069	0.182	0.767	2.636
1995/ 1996	0.703	1.028	0.220	0.902	2.853
1996/ 1997	0.638	1.044	0.156	0.756	2.594
1997/ 1998	0.503	0.705	0.116	0.894	2.218
1998/ 1999	0.502	0.862	0.148	0.833	2.396
1999/ 2000	0.497	0.855	0.153	0.917	2.421
2000/ 2001	0.480	0.823	0.141	0.823	2.266
2001/ 2002	0.557	0.958	0.190	1.098	2.803
2002/ 2003	0.670	1.178	0.208	1.480	3.536
2003/ 2004	0.668	1.024	0.161	1.062	2.915
2004/ 2005	0.509	0.784	0.132	0.824	3.249
2005/ 2006	0.520	0.786	0.115	0.688	2.110
2006/ 2007	0.760	1.047	0.157	1.035	2.999

Alcohol distilleries are considered as one of the largest polluter in Thailand. The effluents from such industry cause color problems, slime growth, thermal impacts, scum formation, and loss of aesthetic beauty in the environment. They also increase the amount of toxic substances in the water, causing death to the zooplankton and fishes, as well as profoundly affecting the terrestrial ecosystem.

The increasing public awareness of the fate of this pollutant and stringent regulations established by the various governmental authorities are forcing the industry to treat effluents to the required compliance level before discharging them into the environment.

2.2 Alcohol production from sugarcane molasses

Alcohol can be produced from a wide range of feedstock. These include sugar-based (sugarcane and beet molasses, cane juice), starch-based (corn, wheat, cassava, rice, barley) and cellulosic (crop residues, sugarcane bagasse, wood, municipal solid wastes) materials.

The production of alcohol in distilleries based on sugarcane molasses constitutes a major industry in Asia and South America. The world's total production of alcohol from sugarcane molasses is more than 13 millions m^3/year.

The manufacture of alcohol in distilleries consists of four main steps as follow: feed preparation, fermentation, distillation and packaging (Figure 1).

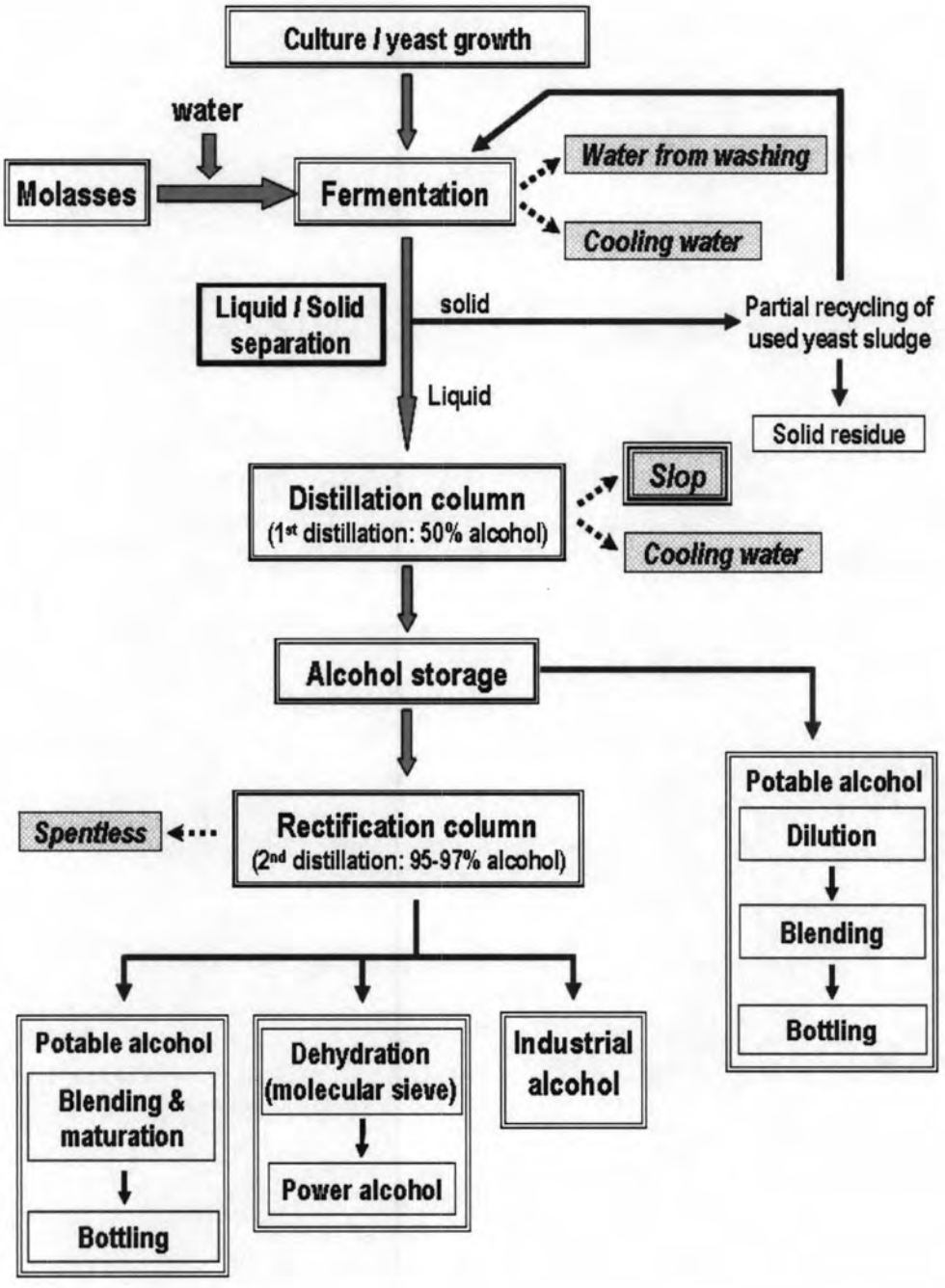

Figure 1 Alcohol manufacturing process (modified from Satyawali and Balakrishnan, 2008)

2.2.1 Feed preparation

Molasses is diluted to about 20–25 brix in order to obtain desired sucrose level and pH is adjusted to below 5 using sulfuric acid before fermentation. It is then supplemented with assimilable nitrogen source like ammonium sulfate or urea. If necessary, it is also supplemented with phosphate.

The composition of molasses varies with the variety of cane, the agro climatic conditions of the region, sugar manufacturing process, handling and storage (Godbole, 2002). Table 2 summarizes the chemical composition of sugarcane molasses.

Table 2 Composition of sugarcane molasses (Godbole, 2002; Chen and Chou, 1993).

Property	Sugarcane molasses	
	Godbole (2002)	Chen and Chou (1993)
Brix (%)	79.5	85 - 92
Specific gravity	1.41	1.38 - 1.52
Total solids (%)	75.0	75 - 88
Total sugar (%)	44 - 60	50 - 90
Crude protein (%)	3.0	2.5 – 4.5
Total fat (%)	0.0	0.0
Total fiber (%)	0.0	0.0
Ash (%)	8.1	7 - 15
Calcium (%)	0.8	NR
Phosphorus (%)	0.08	NR
Potassium (%)	2.4	NR
Sodium (%)	0.2	NR
Chlorine (%)	1.4	NR
Sulfur (%)	0.5	NR

NR: Not reported.

2.2.2 Fermentation

In general, yeast culture (*Saccharomyces cerevisiae*) is prepared in the laboratory and propagated in a series of fermenters. The feed is inoculated with about 10% by volume of yeast inoculum. This is an anaerobic process carried out under controlled conditions of temperature and pH. Sucrose is broken down to ethanol and carbon dioxide. Fermentation can be carried out in either batch or continuous mode. Ethanol accumulates up to 8–10% in the fermented mash. The fermented mash is then distilled, fractionated and rectified after the removal of yeast sludge (Pathade, 2003). The residue of the fermented mash which comes out as liquid waste is termed as distillery slop or spentwash (Pathade, 2003; Singh et al, 2004; Nandy et al, 2002).

In literature, apart from yeasts, a bacterial strain, *Zymomonas mobilis*, has been demonstrated as a potential candidate for ethanol production (Chandraraj and Gunasekaran, 2004).

2.2.3 Distillation

Distillation is a two-stage process and is typically carried out in a series of bubble cap fractionating columns. The first stage consists of the distillation column and is followed by rectification columns. The cell free fermentation broth is preheated to about $90^{o}C$ by heat exchange with the effluent ("slop") and then sent to the degasifying section of the distillation column. Here, the liquor is heated by live steam and fractionated to give about 50% alcohol. The wastewater discharge from the distillation column is the slop. The alcohol vapors are led to the rectification column where by reflux action, 95 -97% alcohol is tapped, cooled and collected. The condensed water from this stage, known as "spentlees" is usually pumped back to the distillation column.

2.2.4 Packaging

Rectified spirit (95-97% ethanol by volume) is marketed directly for the manufacture of chemicals such as acetic acid, acetone, oxalic acid and absolute alcohol. Denatured ethanol for industrial and laboratory uses typically contains 60–95% ethanol as well as between 1% to 5% each of methanol, isopropanol, methyl isobutyl ketone (MIBK), ethyl acetate, etc. (Skerratt, 2004).

For beverages, alcohol is matured and blended with malt alcohol and diluted to the requisite strength. Then it is bottled appropriately in a bottling plant.

Anhydrous ethanol for fuel-blending applications ("power alcohol") requires concentration of ethanol to 99.5% purity.

2.3 Molasses-based distillery wastewaters generation and characteristics

Alcohol production from molasses generates large volumes of high strength wastewater that is of serious environmental concern. The aqueous effluent stream from distilleries known as sugarcane molasses wastewater is approximately 12–15 times the volume of the produced alcohol. The wastewater from distillery is characterized by extremely high chemical oxygen demand (COD) (80,000–100,000 mg/l) and biochemical oxygen demand (BOD) (40,000–50,000 mg/l). However, the amount and the characteristics of the sugarcane molasses wastewater are highly variable and dependent on the raw material used and on the ethanol production process (Pant and Adholeya, 2007; Satyawali and Balakrishanan, 2008). Washing water used to clean the fermenters, cooling water and boiler water further contribute to its variability (Pant and Adholeya, 2007). The main source of wastewater is the distillation step wherein large volumes of dark brown effluent (termed as spentwash, stillage, slop or vinasse) is generated with a temperature range of 70–80 °C, acidic (low pH), and with high concentration of organic materials and solids (Yeoh, 1997; Nandy et al., 2002).

Apart from high organic content, distillery wastewater also contains nutrients in the form of nitrogen, phosphorus and potassium (Mahimairaja and Bolan, 2004) that can lead to eutrophication of water bodies. Further, its dark color leads to widespread damage to aquatic life. Table 3 summarizes the typical characteristics of distillery slop generated in Thai distilleries using molasses.

Table 3 Quantities and characteristics of distillery slop generated in 32 Thai distilleries (The Excise Department, 1983).

Parameters	Values	
	Range	Average
Discharge volume (m^3/ day)	23 - 400	90
pH	2.3 – 5.5	3.7
Temperature (°C)	53 – 100	88.6
BOD_5 (mg/l)	17,500 – 45,000	27,475
COD (mg/l)	56,970 – 193,600	118,100
COD/BOD_5	1.90 – 7.67	4.3
Suspended solid (SS) (mg/l)	5,240 – 23,830	11,319
Total solid (TS) (mg/l)	36,280 – 123,640	75,830
Total volatile solid (TVS) (mg/l)	30,280 – 59,220	58,520

Table 3 Quantities and characteristics of distillery slop generated in 32 Thai distilleries (The Excise Department, 1983). (continued)

Parameters	Values	
	Range	Average
Total nitrogen (mg/l)	40 – 2,160	940
Phosphate (mg/l)	24 – 380	115
Potassium (mg/l)	2,300 – 8,900	4,760
Sulfate (mg/l)	1,820 – 5,160	3,720

Source: Thailand Institute of Scientific and Technological Research (TISTR)

The recalcitrant nature of wastewater from sugarcane molasses is due to the presence of the dark brown colorants, which are biopolymeric colloidal materials that are negatively charged. Except caramel ,all colorants contain phenolic groups which contribute to their formation. Infrared spectra of alkaline degradative products indicate the presence of high molecular weight amino acids. It has been suggested that most of the phenolic colorants are derived from benzoic and cinnamic acid that are precursors of flavanoids, the yellow pigments of the plants, responsible for color formation. The phenolic acids which form colored complexes with iron or get oxidized to polymeric colorants are o-hydroxy or o-dihydroxy acids (Mane et al., 2006). During heat treatment, Maillard reaction takes place resulting in formation of melanoidins, one of the final products of the Maillard reaction (Pant, 2007; Singh et al., 2004; Mohana et al., 2007; Kumar et al., 1997). Apart from melanoidins, the other recalcitrant compounds present in the waste are caramel, different products of sugar decomposition, anthocyanins, tannins and different xenobiotic compounds (Pandey et al., 2003). The unpleasant odor of the effluent is due to the presence of skatole, indole and other sulfur compounds, which are not decomposed by yeast during distillation (Sharma et al., 2007).

2.4 Melanoidins

Melanoidins are dark brown to black colored natural condensation products of sugars and amino acids, they are produced by non-enzymatic browning reactions known as Maillard reactions (Plavsic et al., 2006). Naturally melanoidins are widely distributed in food (Painter, 1998), drinks and widely discharged in huge amount by various agro-based industries especially from distilleries using sugarcane molasses and fermentation industries as environmental pollutants (Kumar and Chandra, 2006;

Gagosian and Lee, 1981). The structure of melanoidins is still not completely understood but it is assumed that it does not have a definite structure as its elemental composition and chemical structures largely depend on the nature and molar concentration of parent reacting compounds and reaction conditions as pH, temperature, heating time and solvent system used (Ikan et al., 1990; Yaylayan and Kaminsky, 1998).

Food and drinks such as bakery products, coffee and beer having brown colored melanoidins exhibited antioxidant, antiallergenic, antimicrobial and cytotoxic properties as *in vitro* studies have revealed that products from Maillard reaction may offer substantial health promoting effects. They can act as reducing agents, metal chelators and radical scavengers(Borrelli et al., 2003; Plavsic et al., 2006). Besides, these health-promoting properties, melanoidins also have antioxidant properties, which render them toxic to many microorganisms such as those typically present in wastewater treatment systems (Kumar et al., 1997). The resistance 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.

2.4.1 Melanoidin formation pathway

The formation of melanoidins is the result of polymerization reactions of highly reactive intermediates formed during Maillard reaction. A wide range of reactions takes place, including cyclizations, dehydrations, retroaldolizations, rearrangements, isomerizations and further condensations, which lead to the formation of brown nitrogenous polymers and copolymers, known as melanoidins. The molecular weight of colored compounds increases as browning proceeds.

The complexity of Maillard reaction has been extensively studied during recent years and new important pathways and key intermediates have been established (Martins et al., 2001). A scheme of Maillard reaction is shown in Figure 2. Melanoidins are recognized as being acidic compounds with charged nature. With increasing reaction time and temperature, the total carbon content increases, thus promoting the unsaturation of the molecules. The color intensity increases with the polymerization degree. The degree of browning, usually measured via absorbance at 420 nm, is often used to follow the extent of Maillard reaction.

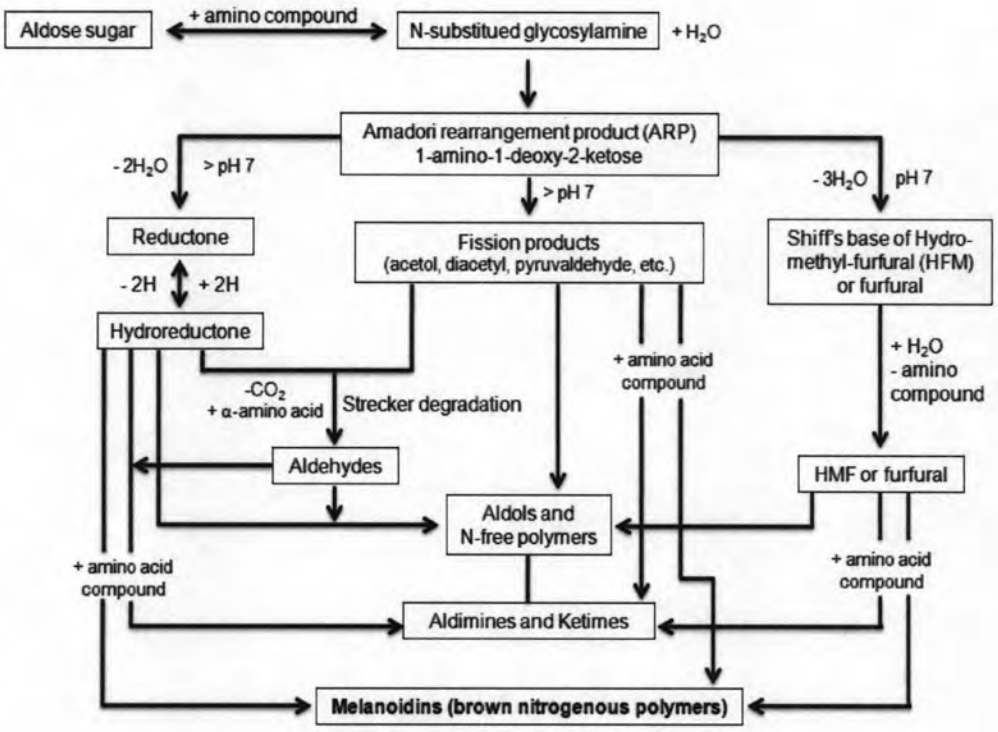

Figure 2 Scheme of Maillard reaction (Martins et al., 2001).

Hayase et al. (1982) reported the formation of a C_3 sugar fragment in early stages of browning reaction between sugar and amines or amino acids, which was identified as methylglyoxal dialkylamine. Fay and Brevard (2004) studied the initial steps of Maillard reaction and reported that the first stable intermediate compound produced in the initial stages of Maillard reaction were Amadori compounds, *N*-substituted 1-amino-1-deoxyketoses, representing an important class of Maillard intermediates, which were produced during the initial phases of Maillard reaction by Amadori rearrangement of corresponding *N*-glycosyl amines.

This type of rearrangement was named after Mario Amadori who was the first to demonstrate the condensation of D-glucose with an aromatic amine. This reaction would yield two structurally different isomers, *N*-substituted glycosyl-amine, which was more labile than the other, N-substituted 1-amino-1-deoxy-2-ketose, towards hydrolysis. Hence, these intermediates of Maillard reaction were termed as Amadori compounds. It has been suggested that marine humic and fulvic acids are formed by

the condensation of sugars with amino acids or proteins via Maillard reaction. Further, the results indicate that various heterocyclic moieties are the main building blocks of humic substances rather than aromatic benzenoid structures (Ikan et al., 1992). Hayashi and Namiki (1986) have also observed that C_3 imine formation followed the pattern of C_2 imine formation, and was well correlated to decrease in the amount of glucosylamine and an increase in the formation of Amadori products. Reaction of Amadori products with n-butylamine rapidly produced C_3 compound in a manner similar to that of glucose-*n*-butylamine system. These results indicated the possibility of participation of Amadori products in the formation of C_3 compound. In spite of large research work done on the Maillard reaction, many parts as mechanism of melanoidins formation at later final stages of Maillard reaction are still obscure. However, the proposed mechanisms reviewed above present a clear picture of melanoidins formation through Maillard amino–carbonyl reaction.

2.4.2 Structure of melanoidin polymer

The elucidation of the chemical structure of melanoidins is difficult due to the complexity of the Maillard reaction. Kato and Tsuchida (1981) proposed a major repeating unit for melanoidins prepared from glucose and butylamine (pH 5.0–6.5). The structure is useful for explaining the great increase of the reductone content of melanoidins on heat treatment under anaerobic conditions. However, changing reaction conditions play an important role in the fundamental structure of melanoidins. This means that it cannot be assumed that melanoidins have a regular composition with repeating units. For this reason, Cämmerer and Kroh (1995) proposed a general structure for melanoidins prepared from monosaccharides and glycine. The chemical structure of investigated melanoidins aforementioned above is shown in Figure 3.

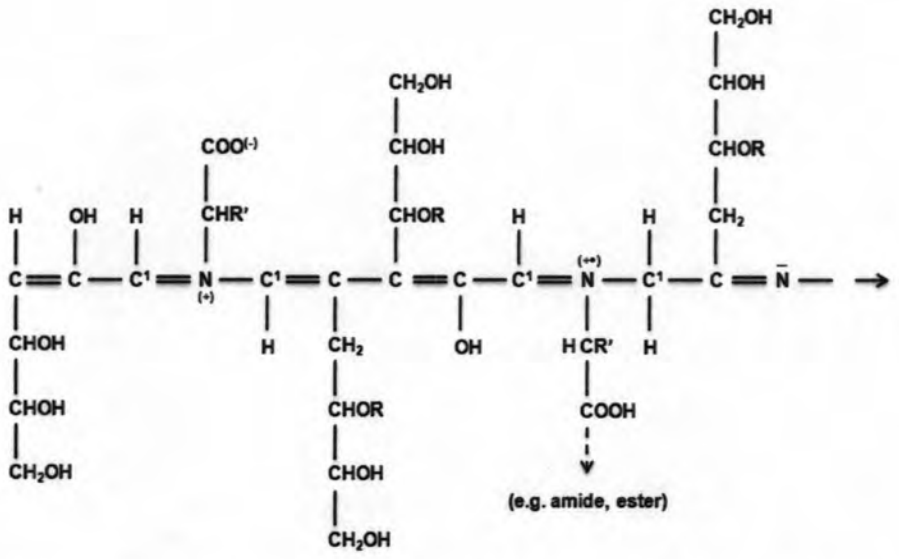

Figure 3 Proposal for the general structure of the melanoidin polymer (Cämmerer and Kroh, 1995). R:H or saccharides. R': side chain of amino acid.

The basic structure is formed by α-dicarbonyl Maillard reaction intermediates, partially branched by amino compounds and with many reactive centers that make possible further decarboxylation and dehydration reactions. The structure of the real melanoidins is likely to be a result of different reactions from the basic framework. Yaylayan and Kaminsky (1998) isolated a brown nitrogen-containing polymer formed in the Maillard mixture. The structure was consistent with the one proposed by Cämmerer and Kroh (1995). This polymer exhibited a strong absorption band at 1607 cm^{-1} in the FTIR spectrum, attributed to extensive conjugation. Pyrolysis of the isolated polymer produced typical Amadori products, such as pyrazines, pyrroles, pyridines and furans. Cämmerer et al., (2002) have recently suggested a new model of a basic skeleton for melanoidins formed from carbohydrates and amino acid (Figure 4)

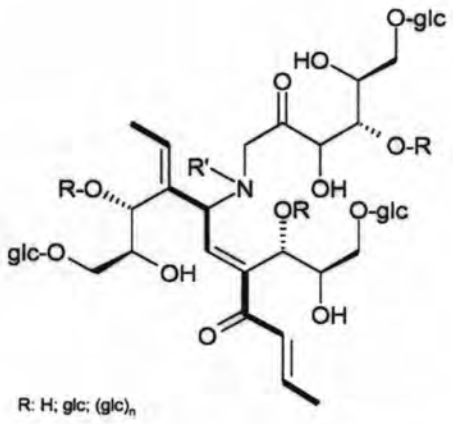

Figure 4 Basic melanoidin structure formed from carbohydrates and amino acid (Cämmerer et al., 2002).

Although the chemical structure of melanoidins is not clearly understood, but some part of the chemical structure of model melanoidins have recently been elucidated by different spectral studies such as ^{1}H NMR, CP-MAS NMR, etc. (Ikan et al., 1990; Ikan et al., 1992; Larter and Douglas, 1980). The chemical investigations have revealed that natural and synthetic melanoidins both have similar elemental (CHON) compositions, spectroscopic properties and electrophoretic mobilities at various pH values (Migo et al., 1997; Ikan et al., 1990; Ikan et al., 1992). However, the nitrogen contents, acidities and electrophoretic behavior of the polymers all reflect functional group distributions inherited from the amino acids (Hedges, 1978).

In spite of these studies, the melanoidins chromophore has not been yet identified. Hence, the chemical structure of the so-called melanoidin is still not clear but probably it does not have a definite one and there exists various types of melanoidins differing in structure depending on parent reactants and reaction conditions as pH, temperature and reaction time. Moreover, it further needs intensive investigations with more refined recent and advanced techniques for the elucidation of chromophore structure to deduce the main skeleton of melanoidin polymer.

2.5 Environmental hazards of molasses-based distillery wastewaters

Sugarcane molasses-based distillery wastewaters 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 (Kumar et al., 1997). The highly colored components of the molasses

wastewater reduce sunlight penetration in rivers, lakes or lagoons which in turn decrease both photosynthetic activity and dissolved oxygen concentration affecting aquatic life. Kumar et al. (1995) evaluated the toxic effect of distillery effluent on common guppy, *Lesbistes reticulates* and observed remarkable behavioural changes with varying effluent concentration. Kumar and Gopal (2001) reported hematological alterations in fresh water catfish, *Channa punctatus*, exposed to distillery effluents. Saxena and Chauhan (2003) 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 (2003) observed concentration dependent toxicity of distillery effluent on the fresh water crab, *Barythephusa guerini*.

Disposal of sugarcane molasses wastewater on land is equally hazardous to the vegetation. It was reported to reduce soil alkalinity and manganese availability, thus inhibiting seed germination (Kumar et al., 1997). Kannan and Upreti (2008) reported highly toxic effects of raw distillery effluent on the growth and germination of *Vigna radiata* seeds even at low concentration of 5% (v/v). 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, etc. due to leaching down of the organic and inorganic ions (Jain et al., 2005). Juwarkar and Dutta (1990) evaluated the impact of application of distillery effluent on soil microflora. Irrigation with raw distillery effluent resulted in low overall bacterial and actinomycetes count. Nitrogen fixing bacteria *Rhizobium* and *Azotobacter* reduced considerably. However, population of fungi increased. Anaerobically treated effluent also showed similar reduction as previously mentioned with bacteria but not as much as that of the raw effluent.

2.6. Treatment technologies for sugarcane molasses wastewater

Several technologies have been explored for reducing the pollution load of sugarcane molasses wastewater. Majority of these methods decolorize the effluent by either concentrating the color into the sludge or by breaking down the colored molecules. These treatment technologies are discussed in detail in the following section.

2.6.1 Treatments based on physicochemical methods

2.6.1.1 Adsorption

Among the physicochemical treatment methods, adsorption on activated carbon is widely employed for removal of color 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 decolorization of molasses wastewater include adsorption on commercial as well as indigenously prepared activated carbons (Satyawali and Belakrishanan, 2008). Decolorization of synthetic melanoidins using commercially available activated carbon as well as activated carbon produced from sugarcane bagasse was investigated (Satyawali and Balakrishnan, 2007). The adsorptive capacity of the different activated carbons was found to be quite comparable.

Pendyal et al. (1999) found twenty-four granular activated carbons (GACs) made from mixtures of four binders (coal tar, sugarcane molasses, sugar beet molasses, and corn syrup) and three agricultural by-products (rice hulls, rice straw, and sugarcane bagasse) which were evaluated for their ability to remove sugar colorants (molasses color removal and sugar decolorization). These properties were compared to those of two commercial reference carbons. GACs made from sugarcane bagasse, in general, possessed the best ability to remove sugar colorants and were closest to the reference carbons in this regard. In fact, the four highest ranked GACs all used bagasse as a feedstock along with four different binders. Therefore, the ability to remove sugar colorants appears to be by-product dependent with the binder playing a minor role.

2.6.1.2. Oxidation processes

Ozone is a powerful oxidant for water and waste water 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 2003).

Oxidation by ozone could achieve 80% decolorization for biologically treated molasses wastewater 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 (Alfafara et al., 2000; Peña et al., 2003). Ozone in combination with UV radiation enhanced molasses wastewater degradation in terms of COD; however, ozone with hydrogen peroxide showed only marginal reduction even on a very dilute effluent (Beltran et al., 1997). Samples exposed to 2 h ultrasound pre-treatment

displayed 44% COD removal after 72 h of aerobic oxidation compared to 25% COD reduction shown by untreated samples.

The Fenton's oxidation technology is based on the production of hydroxyl radicals .OH, which has 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 (Fe^{2+} or Fe^{3+}) which produces hydroxyl radicals which ultimately leads to decolorization of the effluent (Pala and Erden, 2005)

Another option is photo-catalytic oxidation that has been studied using solar radiation and TiO_2 as the photocatalyst (Kulkarni, 1998). 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.

2.6.1.3 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 (Ecologix Environmental system, LLC, 2008)

2.6.1.4 Membrane treatment

Pre-treatment of molasses wastewater with ceramic membranes prior to anaerobic digestion was reported to halve the COD from 36,000 to 18,000 mg/l (Chang et al., 1994). The total membrane area was 0.2 m^2 and the system was operated at a fluid velocity of 6.08 m/s with 0.5 bar transmembrane pressure.

Electrodialysis has been explored for desalting molasses wastewater using cation and anion exchange membranes resulting in 50–60% reduction in potassium content (de Wilde, 1987). Vlyssides et al. (1997) reported the treatment of vinasse from beet molasses by electro dialysis using a stainless steel cathode, titanium alloy anode and 4% w/v NaCl as electrolytic agent. Up to 88% COD reduction at pH 9.5 was obtained. However, the COD removal percentage decreased at higher wastewater feeding rates.

In a recent study, Nataraj et al. (2006) reported pilot trials on distillery spent wash using a hybrid nanofiltration (NF) and reverse osmosis (RO) process. NF was primarily effective in removing the color and colloidal particles accompanied by 80 and 45% reduction in total dissolved solids and chloride concentration, respectively, at an optimum feed pressure of 30–50 bar.

2.6.1.5 Evaporation and combustion

Molasses wastewater containing 4% solids can be concentrated to a maximum of 40% solids in a quintuple-effect evaporation system with thermal vapor recompression (Bhandari et al., 2004; Gulati, 2004). The condensate with a COD of 280 mg/l can be used in fermenters. The concentrated mother liquor is spray dried using hot air at 180^{o}C to obtain a desiccated powder. The powder is typically mixed with 20% agricultural waste and burnt in boiler. Combustion is also an effective method of on-site vinasse disposal as it is accompanied by production of potassium-rich ash that can be used for land application (Cortez and Perèz, 1997).

2.6.1.6 Other treatments

Pikaev (2001) applied radiation technology for treatment of distillery waste. The study involved a combined treatment of electron beam 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 pretreatment step and the results indicated that ultrasound treatment enhanced the biodegradability of the distillery waste water (Sangave and Pandit, 2004). Chaudhari et al. (2008) proposed a novel catalytic thermal pretreatment or catalytic thermolysis to recover the majority of its energy content with consequent COD and BOD 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 physicochemical methods such as adsorption, coagulation–flocculation, and oxidation processes like Fenton's oxidation, ozonation, electrochemical oxidation using various electrodes and electrolytes, nanofiltration, 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 physiochemical methods which are summarized in Table 4 below.

Table 4 Summary of various physicochemical treatments used for the treatment of sugarcane molasses-based distillery wastewaters and their efficiency

Treatment	COD removal (%)	Color removal (%)	References
Adsorption			
Chitosan, a biopolymer was used as anion exchanger	99	98	Lalvo et al., 2000
Chemically modified bagasse			Mane et al., 2006
DEAE bagasse	40	51	
CHPTAC bagasse	25	50	
Activated carbon prepared from agro industrial waste			Satyawali and Balakrishanan, 2008
Phosphoric acid carbonized bagasse	23	50	
Commercially available activated carbon			
AC (ME)	76	93	
AC (LB)	88	95	
Coagulation–flocculation			
Flocculation of synthetic melanoidins was carried out by various inorganic ions			Migo et al., 1997
Polyferric hydroxysulphate (PFS)	NR	95	
Ferric chloride ($FeCl_3$)	NR	96	
Ferric sulphate ($Fe_2(SO_4)_3$)	NR	95	
Aluminium sulphate ($Al_2(SO_4)_3$)	NR	83	
Calcium oxide (CaO)	NR	77	
Calcium chloride ($CaCl_2$)	NR	46	

Table 4 Summary of various physicochemical treatments used for the treatment of sugarcane molasses-based distillery wastewaters and their efficiency. (Continued)

Treatment	COD removal (%)	Color removal (%)	References
Oxidation processes			
Fenton's oxidation	88	99	Pala and Erden, 2005
Ozonation	15–25	80	Pena et al., 2003
Electrochemical oxidation			
Graphite electrodes	80.6	95.6	Manisankar et al., 2003
Lead dioxide coated on titanium	90.8	98.5	
Ruthenium dioxide coated on titanium	92.1	99.5	
Electrocoagulation and electro Fenton	92.6	–	Yavuz, 2007
Membrane technologies			
Reverse osmosis	99.9	–	Nataraj et al., 2006
Nanofiltration	97.1	100	

NR: Not reported.

Physicochemical 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 costs and sensitivity to variable water input. 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.

2.6.2 Treatments based on biological methods

Biological treatment of molasses wastewater is either aerobic or anaerobic but in most cases a combination of both is used. Anaerobic treatment is an accepted practice and various high rate reactor designs have been tried at pilot and full scale operation. Aerobic treatment of anaerobically treated effluent using different microbial populations has also been explored. Majority of biological treatment technologies remove color by either concentrating the color into sludge or by partial or complete breakdown of the color molecules. These methods are discussed in detail in the following section.

2.6.2.1 Anaerobic systems

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 (Jain et al., 1990). 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 (Nandy et al., 2002). Further, low nutrient requirements and stabilized sludge production are other associated benefits (Jiménez et al., 2004). However, the performance and treatment efficiency of anaerobic process can be influenced both by inoculum source and feed pre-treatment. 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 (Patel and Madamwar, 2000).

Anaerobic lagoon

Anaerobic lagoons are the simplest choice for anaerobic treatment of molasses wastewater. Rao (1972) 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 (Singh et al., 2004).

Conventional anaerobic systems

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 (Pathade, 2003). The HRT in CSTR-type reactor is determined by the specific growth rate of the slowest growing microorganism in the system. This generally means that very high HRT values are required to achieve an acceptable level of degradation. The high HRT values make the CSTR concept less feasible and unattractive for treatment of the wastewaters (Kleerebezem and Macarie, 2003).

Anaerobic batch reactors

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 (Ruiz et al., 2002).

Banerjee and Biswas (2004) 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.

Anaerobic fixed film reactors

In fixed film reactors, the reactor has a biofilm support structure (media) for biomass attachment. Figure 5 shows the schematic representation of an anaerobic fixed film reactor. 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 (Rajeshwari et al, 2000). Amongst numerous

anaerobic reactors developed for biomethanation, anaerobic fixed film reactors (AFFR) have emerged as the most popular one compared to other reactors due to availability of large biomass in the reactor (Patel and Madamwar, 2002). The nature of the media used for biofilm attachment has a significant effect on reactor performance. A wide variety of materials like glass bead, red drain clay, sand and a number of different plastics and porous materials such as needle punched polyesters, polyureathane foam and sintered glass (Perez, 1997), waste tire rubber (Borja, 1996), poly(acrylonitrile–acrylamide) (Lalov, 2001), corrugated plastic (Perez-Garcia, 2005), etc., have been used as non-porous support media at laboratory as well as pilot-scale

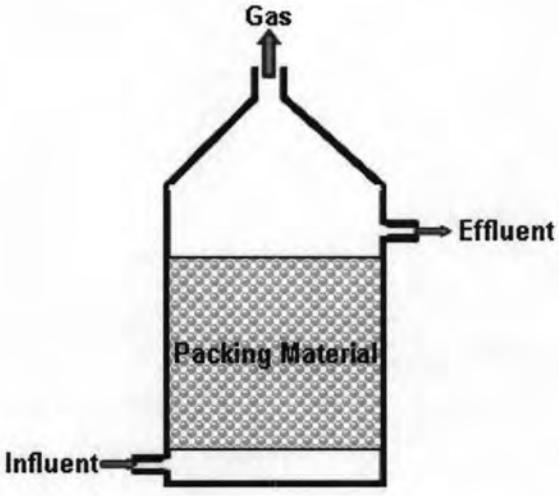

Figure 5. Schematic diagram of anaerobic fixed film reactor (modified from Kansal et al., 1998).

In another study, Perez-Garcia et al., (2005) studied the influent pH conditions in fixed film reactors for anaerobic thermophilic treatment of wine distillery wastewaters. They showed that the pH of the influent influenced the performance of the biodegradation process and the depurative efficiency was higher with alkaline influent. The operation with acidic influent allowed the reactor to operate at OLR around 5.6 kg COD m^{-3} d^{-1} (HRT: 1.5 days), maintaining total COD removals of 77.2%; the operation with alkaline influent allowed total COD removals of 76.8% working at OLR around 10.5 kg COD m^{-3} d^{-1}. The greatest efficiency of substrate removal was 87.5% for OLR 3.2 kg COD m^{-3} d^{-1} and HRT of 4 days operating with alkaline influent. Therefore, the operation with alkaline influent implicates higher levels of purifying efficiency for similar organic load rate.

Acharya et al., (2008) performed a comparative study of low cost packing materials for the treatment of distillery spent wash using anaerobic fixed film reactors. Coconut coir was found to be the best supporting material, as the system supported the treatment at very high organic loading rate of 31 kg COD m^{-3} d^{-1} with 50% COD reduction. Charcoal and Nylon fibers were other packing materials used in the study. Charcoal was able to retain the active biomass at the OLR of 15.5 kg COD m^{-3} d^{-1} resulting in more than 60% COD reduction whereas nylon fibers failed to support the biofilm development even at higher HRT and lower OLR.

Upflow anaerobic sludge blanket (UASB) reactors

The UASB process has been successfully used for the treatment of various types of wastewaters (Lettingar and Holshoff Pol, 1991). 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 (Fang et al., 1994). These granules consist of aggregation of anaerobic bacteria, self immobilized into compact forms. This enhances the settelability of biomass and leads to an effective retention of bacteria in the reactor (Akunna and Clark, 2000). Particularly attractive features of the UASB reactor design include its independence from mechanical mixing, recycling of sludge biomass (Kalyazhnyi et al., 1997) and ability to cope up with perturbances caused by high loading rates and temperature fluctuations (Sharma and Singh, 2002). The schematic representation of an UASB reactor is shown in Figure 6. The UASB technology is well suited for high strength distillery wastewaters when the process has been successfully started up and is in stable operation.

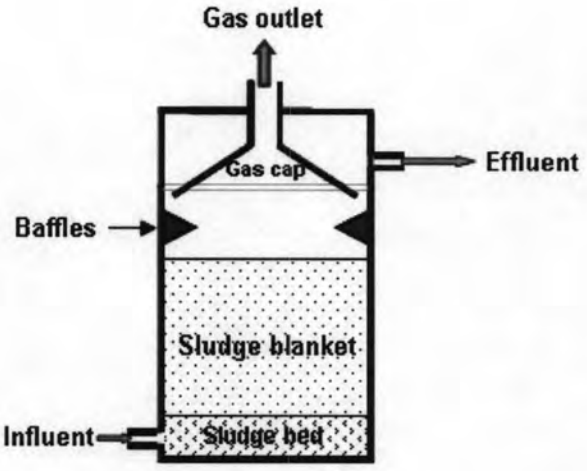

Figure 6 Schematic diagram of anaerobic UASB reactor (modified from Kansal et al., 1998).

Potable of malt whisky distillery, a liquid waste product from the malt whisky industry, was treated in a laboratory scale UASB reactor, pH control was of interest to attain a high COD reduction (Goodwin and Stuart, 1994). There is normally a rise in the pH due to ammonia production during the process of digestion. The maximum loading rate for a stable operation was 15 kg COD m^{-3} d^{-1} at a retention time of 2.1 days.

Successful operation of the UASB reactors for treating distillery waste at psychrophilic temperatures (4–10 °C) was also studied by operating one and two-stage UASB reactors. The organic loading rate varied from 4.7 to 1.3 g COD at HRT of 6–7 days for one-stage reactor and 2 days for the two-stage reactor. The average total COD removal for vinasses waste waters was 60% in the one-stage reactor and 70% in the two-stage reactor. Therefore, application of high recycle ratios is essential for enhancement of UASB pretreatment under psychrophilic conditions (Kalyazhnyi et al., 2001).

Uzal et al., (2003) investigated an anaerobic treatment of whisky distillery waste in two-stage UASB reactors and concluded that the system worked efficiently even at OLRs as high as 39 kg COD m^{-3} d^{-1} resulting in 95–96% COD reduction.

Anaerobic fluidized bed reactors

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. The schematic representation of an anaerobic fluidized bed reactor is shown in Figure 7.

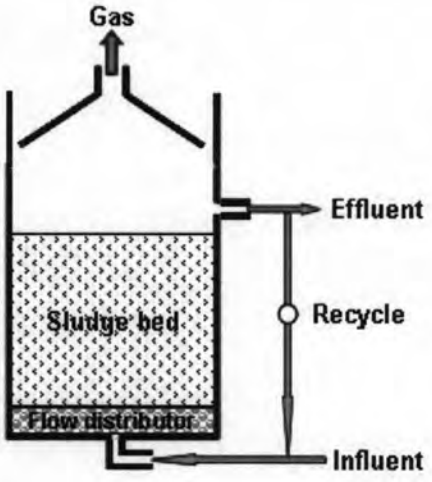

Figure 7 Schematic diagram of anaerobic fluidized bed reactor (modified from Kansal et al., 1998).

Kida et al., (1995) studied the biological treatment of Shochu distillery wastewater using an anaerobic fluidized bed reactor. The maximum loading rate of 22 kg TOC m^{-3} d^{-1} could be achieved by the addition of nickel, cobalt and diluting the waste. This resulted in 70% TOC reduction.

Ability of anaerobic fluidized bed reactor to treat high strength wastewaters like distillery waste under thermophilic temperatures was studied by Perez et al., (1997). The results showed that AFB systems can achieve over 82.5% COD reduction at a COD loading rate of 32.3 kg COD m^{-3} d^{-1} corresponding to HRT of 0.46 day. The highest efficiency of substrate removal was 97% for an organic loading rate of 5.9 kg COD m^{-3} d^{-1} and HRT of 2.5 days.

2.6.2.2 Aerobic systems

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 (Nandy et al., 2002). Therefore, aerobic treatment of sugarcane molasses wastewater has been mainly attempted for the decolorization 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 wherein 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 (Torrijos and Moletta, 1997). 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. Thus several pure cultures of fungi, bacteria and algae have been investigated specifically for their ability to decolorize the sugarcane molasses wastewater as discussed earlier. Pure bacterial and fungal cultures have been studied to develop bioprocess for melanoidins decolorization of molasses wastewater. However, the performance of fungal decolorization was limited by long growth cycle and moderate decolorization rate. In contrast, the bacterial decolorization is normally faster, but it may require a mixed community to decolorize melanoidins though a combined metabolic mode of individual culture. The bacterial

consortium seems to be more competent for molasses wastewater treatment due to maintenance of microorganism and co-metabolism to enhance the efficiency of melanoidins decolorization. In all instances, it is found necessary to supplement with additional nutrients as well as diluting the effluent for obtaining optimal microbial activity and eventually optimal results. Consequently there is a need to explore more efficient microbes that can decolorize the effluent using it as the sole source of nutrients without much dilution. In addition, these studies are mostly limited to laboratory scale investigations and no pilot/commercial scale operations are reported as yet.

2.6.2.3 Biocomposting process

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 (Kannan and Upreti, 2008).

2.6.2.4 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 (Kumar and Chandra, 2004). Billore et al., (2001) 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 (2004) 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. (2006) which resulted in 98–99% BOD, COD and color reduction after 7 days.

2.6.2.5 Cyanobacterial and algal systems

Cyanobacteria are considered ideal for treatment of molasses wastewater as they, apart from degrading the polymers, also oxygenate waterbodies, 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 decolorization of the effluent (Kalavathi et al., 2001). Patel et al. (2001) have reported 96%, 81% and 26% decolorization of distillery effluent through bioflocculation by *Oscillatoria* sp., *Lyngbya* sp. and *Synechocystis* sp., respectively.

Valderrama et al., (2002) 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.

2.6.2.6 Fungal systems

There is a good number of reports showing the role of fungi in decolorization of melanoidins by adsorption to mycelia as well as the role of ligninolytic enzyme (Raghukumar and Rivonkar, 2001; Vahabzadeh et al., 2004; Watanabe et al., 1982). However, the long growth cycle and spore formation limit the performance of the fungal system

Increasing attention has been directed towards utilizing microbial activity for decolorization of molasses wastewater. Several reports have indicated that some fungi in particular have such a potential (Kumar et al., 1998). One of the most studied fungus having ability to degrade and decolorize distillery effluent is *Aspergillus* such as *Aspergillus fumigatus* G-2-6, *Aspergillus niger*, *Aspergillus niveus*, *Aspergillus fumigatus* $U_{B2}60$ brought about an average of 69–75% decolorization along with 70–90% COD reduction (Ohmomo et al., 1987; Miranda et al., 1996; Jimnez et al., 2003; Shayegan et al., 2004; Angayarkanni et al., 2003; Mohammad et al., 2006).

Treatment of distillery spent wash with ascomycetes group of fungi such as *Penicillium* such as *Penicillium decumbens*, *Penicillium lignorum* resulted in about 50% reduction in color and COD, and 70% phenol removal (Jimnez et al., 2003).

Pant and Adholeya (2007) isolated three cultures of fungi 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 ligninolytic enzymes and decolorized the effluent up to 50%, 47% and 86%, respectively.

Sirianuntapiboon et al., 2004 isolated 205 yeast strains from Thai-fruit samples and screened. Isolate No. WR-43-6 showed the highest decolorization (68.91%) when cultivated at 30 °C for 8 days in a molasses solution containing 2.0% glucose, 0.1% sodium nitrate, and 0.1% KH_2PO_4, the pH being adjusted to 6.0. This potent strain was identified as *Citeromyces* sp. and showed highest removal efficiencies on stillage from an alcohol distillery (U-MWW). The color intensity, chemical oxygen demand (COD) and biochemical oxygen demand (BOD) removal efficiencies were 75%, almost 100 and 76%, respectively. In a periodical feeding system, *Citeromyces* sp. WR-43-6 showed an almost constant decolorization of 60–70% over 8 day feeding of 10% fresh medium. In a replacement culture system, *Citeromyces* sp. WR-43-6 also gave a constant decolorization (about 75%) during four times replacement.

White rot fungi is another group of widely exploited microorganisms in bioremediation of distillery effluent. White rot fungi produce various isoforms of extracellular oxidases including laccases, manganese peroxidases and lignin peroxidases, which are involved in the degradation of lignin in their natural lignocellulosic substrate. This ligninolytic system of white rot fungi is directly involved in the degradation of various xenobiotic compounds and dyes (Wesenberg et al., 2003). Table 5 gives details on different white rot fungi used in decolorization of distillery effluent and the role of different enzymes in the process.

Miyata et al., 2000 reported a white rot fungus, *Coriolus hirsutus*, exhibited a strong ability to decolorize melanoidins in cultures without supplement of nitrogenous nutrients. Addition of peptone to the cultures lowered the ability of the fungus to decolorize melanoidins, but addition of inorganic nitrogens (Ns), ammonium and nitrate did not bring about any marked reduction in the ability. These results suggested an inhibitory effect of organic nitrogens on melanoidin decolorization. Therefore, for enhancing the decolorization of melanoidins in wastewaters by the fungus, activated sludge pretreatment of the wastewaters was expected to be

effective, *i.e.*, activated sludge is capable of converting available organic nitrogens into inorganic nitrogens. To confirm this, waste sludge heat treatment liquor (HTL), wastewater from a sewage treatment plant was pretreated with activated sludge. In practice, pretreatment of HTL under appropriate conditions accelerated the fungal decolorization of HTL. In the pretreated HTL, the fungus was shown to produce a high level of manganese-independent peroxidase (MIP). Addition of Mn(II) to the pretreated HTL caused a further increase in the decolorization efficiency of the fungus and a marked increase in the manganese peroxidase (MnP) activity. Consequently, the increases in MIP and MnP activities were considered to play an important role in the enhanced ability of *C. hirsutus* to decolorize HTL.

Table 5 Microbial cultures employed for treatment of molasses-based distillery wastewaters.

Culture	Treatment	COD removal	Color removal	Enzymes	References
Fungi					
Coriolus sp. No. 20	Synthetic melanoidins solution was decolorized by the fungus	NR	80%	Sorbose oxidase	Watanebe et al., 1982
Phanerochaete chrysosporium	Free cells as well as Ca alginate immobilized cells decolorized the distillery effluent.	NR	85% (free)	NR	Fahy et al., 1997
		NR	59% (immobilized)	NR	
Trametes versicolor	Anaerobically treated distillery effluent supplemented with sucrose and inorganic N sources was decolorized by the culture in shake flask studies	75%	80%	NR	Benito et al., 1997
Phanerochaete chrysosporium	Both strains decolorized and reduced COD of effluent in presence of (3–5%) glucose and 0.1% yeast extract	73%	53.5%	NR	Kumar et al., 1998
Coriolus versicolor		70%	71.5%	NR	
Coriolus hirsutus	Synthetic as well as wastewater melanoidins were decolorized by the fungus in a medium containing glucose and peptone	NR	80%	MiP and MnP and presence of extracellular H_2O_2	Miyata et al., 1998 and Miyata et al., 2000

Table 5 Microbial cultures employed for treatment of molasses-based distillery wastewaters (continued).

Culture	Treatment	COD removal	Color removal	Enzymes	References
Fungi					
Coriolus hirsutus F044917	The fungal culture was immobilized on PUF and used for decolorization of melanoidins present in heat treated liquor	NR	45%	NR	Fujita et al., 2000
Flavodon flavus	Distillery effluent was decolorized using this marine basidiomycetes in presence of 5% glucose.	NR	80%	Glucose oxidase accompanied with hydrogen peroxide	Rughukumar and Rivonkar, 2001; Rughukumar et al., 2004
Penicillium decumbens	Aerobic/Anaerobic biodegradation of beet molasses wastewater.	50.7%	41%	NR	Jimenez et al., 2003
Coriolus versicolor	The cultures were incubated with cotton stalks in vinasses, media under static conditions. No synthetic carbon or nitrogen sources were used.	49%	63%	NR	Kahraman and Yesilada, 2003
Funalia trogii		62	57		
Phanerochaete chrysosporium		57	37		
Pleurotus pulmonarius		34	43		
Phanerochaete chrysosporium 1557	Effect of Veratryl alcohol and Mn (II) on decolorization of distillery effluent was studied.	NR	75%	LiP and MnP	Vahabzadeh et al., 2004

Table 5 Microbial cultures employed for treatment of molasses-based distillery wastewaters(continued).

Culture	Treatment	COD removal	Color removal	Enzymes	References
Fungi					
Phanerochaete chrysosporium ATCC 24725	The fungus was immobilized on different support materials such as PUF and scouring wet and the decolorization was carried out in a RBC	48%	55%	NR	Guimaraes et al., 2005
P. chrysosporium NCIM 1073	The cultures were employed to study the decolorization of molasses in medium containing 2% glucose under static as well as submerged conditions.	Nil	Nil	NR	Thakkar et al., 2006
P. chrysosporium NCIM 1106		NR	82%	LiP and MnP	
P. chrysosporium NCIM 1197		NR	76%	LiP and MnP	
Marine basidiomycetes NIOCC	Experiments were carried out with 10% (v/v) diluted effluent	NR	100%	Laccase and exoploysaccharide produced by the fungus	D'Souza et al., 2006

Table 5 Microbial cultures employed for treatment of molasses-based distillery wastewaters (continued).

Culture	Treatment	COD removal	Color removal	Enzymes	References
Bacteria					
Lactobacillus hilgardii	Decolorization by this bacterial strain when cultivated with melanoidins containing wastewater medium supplemented with 1% of glucose.	NR	28%	NR	Ohmomo et al., 1988
	Decolorization by immobilized cells on calcium alginate.		40%		
Lactobacillus L-2	12.5% diluted wastewater was supplemented with 10 g/l of glucose.	57%	31%	NR	Kumar et al., 1997
Bacillus sp.	The decolorization was studied under anaerobic and thermophilic conditions.	NR	35.5	Decolorization enzyme	Nakajima-Kambe et al., 1999
Aeromonas formicans	Study on predigested distillery effluent.	57%	55%	NR	Jain et al., 2000
Pseudomonas fluorescence	Immobilized cells on porous cellulose carrier.	NR	76%	NR	Dahiya et al., 2001
TA2	Two aerobic bacterial strains isolated from the activated sludge of finally treated distillery effluent were used for the treatment of distillery effluent.	NR	66.67%	NR	Asthana et al., 2001
TA4			63.9%		
Mixed (TA2+TA4)			75%		

Table 5 Microbial cultures employed for treatment of molasses-based distillery wastewaters (continued).

Culture	Treatment	COD removal	Color removal	Enzymes	References
Bacteria					
Pseudomonas putida U	Anaerobically treated distillery spent wash in two stage bioreactor (first stage: *Pseudomonas putida*; second stage: *Aeromonas* strain *Ema*)	44.4%	60%	NR	Ghosh et al., 2002
Aeromonas strain Ema		44%	-		
Bacillus cereus	Experiments were carried out with distillery effluent	81%	75%	NR	Jain et al., 2002
Acetogenic bacteria strain No. BP103	Decolorization by the bacterial culture when cultivated in molasses pigments medium containing glucose 3%, yeast extract 0.5%	NR	76%	Sugar oxidase	Sirianuntapiboon et al., 2004b
Mixture of all six isolates: *Pseudomonas, Enterobacter, Stenotrophomonas, Aeromonas, Acinetobacter* and *Klebsiella*	Study on decolorization of molasses spent wash.	44%	NR	NR	Ghosh et al., 2004

Table 5 Microbial cultures employed for treatment of molasses-based distillery wastewaters (continued).

Culture	Treatment	COD removal	Color removal	Enzymes	References
Bacteria					
Mixed culture of : *Bacillus thuringiensis* *Bacillus brevis* *Bacillus* sp. (MTCC6506)	The decolorization was studied with 4 types of synthetic melanoidins as follow: GGA (glucose-glutamate-acid) GAA (glucose-aspartic-acid) SGA (sucrose-glutamate-acid) SAA (sucrose-aspartic-acid)	 53.91% 36.13% 63.39% 54.51%	 45.12% 28.88% 50.56% 46.08%	Sugar oxidase and peroxidase	Kumar and Chandra, 2005
Microbacterium hydrocarbonoxydans *Achromobacter xylosoxidans* *Bacillus subtilis* *Bacillus megaterium* *Bacillus anthracis* *Bacillus licheniformis* *Achromobacter xylosoxidans* *Achromobacter* sp. *Bacillus thuringiensis* *Bacillus licheniformis* *Bacillus subtilis* *Staphylococcus epidermidis* *Pseudomonas migulae* *Alcaligens faecalis* *Bacillus cereus*	All the fifteen isolates grown on effluent supplemented medium as a sole carbon source	86.14%	75.5%	NR	Chaturvedi et al., 2006

Table 5 Microbial cultures employed for treatment of molasses-based distillery wastewaters (continued).

Culture	Treatment	COD removal	Color removal	Enzymes	References
Bacteria					
Pseudomonas aerugenosa PA01 *Stenotrophomonas maltophila* *Proteus mirobilis*	The decolorization was studied in effluent with low nutrient medium	67%	51%	NR	Mohana et al., 2007
Yeast					
Citeromyces sp. strain no. WR-43-6	Decolorization was observed on stillage from an alcohol distillery (U-MWW).	99.38%	68.91%	NR	Sirianuntapiboon et al., 2004
Cyanobacteria					
Oscillatoria boryana BDU 92181 (marine cyanobacteria)	Decolorization of pure melanoidins (0.1% w/v)	NR	75%	NR	Kalavathi et al., 2001
	Decolorization of crude pigment in distillery effluent (5% v/v)		60%		
Algae					
Mixed culture of Microalgae: *Chlorella vulgaris* Macrophyte: *Lemna minuscule*	Study with diluted wastewater (diluted wastewater from ethanol production to 10% of original concentration)	61%	52%	NR	Valderrama et al., 2002

2.6.2.7 Bacterial systems

Different bacteria capable of both bioremediation and decolorization of molasses wastewater have been isolated. Table 5 gives details on different bacterial isolates employed in decolorization of molasses-based distillery wastewaters. Some of these studies are also discussed in detail in the following section.

Kumar and Viswanathan (1991) isolated bacterial strains from sewage and acclimatized on increasing concentrations of distillery waste, which were able to reduce COD by 80% in 4–5 days without any aeration and the major products left after the degradation process were biomass, carbon dioxide and volatile acids.

Toshiaki et al., 1999 could screen various molasses wastewater-decolorizing microorganisms under thermophilic and anaerobic conditions. Strain MD-32, newly isolated from a soil sample, was selected as the candidate strain. From taxonomical studies, this strain belonged to the genus *Bacillus*, most closely resembling *B. smithii*. The strain decolorized 35.5% of molasses pigment within 20 days at 55°C under anaerobic conditions, but no decolorization activity was observed when cultivated aerobically. At all the concentrations tested, molasses pigment was effectively decolorized by MD-32, with decolorization yields of approximately 15% within 2 days. The molecular weight distribution as determined by gel filtration chromatography revealed that the decolorization of molasses pigment by the isolated strain is accompanied by a decrease in not only small molecules but also large ones.

Acetogenic bacteria strain No.BP103 could decolorize 73.5% of molasses pigments in molasses wastewater supplemented with glucose, yeast extract, and basal mineral salts whereas the decolorization of this strain was decreased to only 9.75% in the absence of supplementary nutrients (Sirianuntapiboon et al., 2004). Nakajima et al. (1999) isolated *Bacillus* sp. which decolorized molasses wastewater up to 35.5% within 20 days at 55 °C under anaerobic conditions. The molecular weight distribution as determination by gel permeation chromatography revealed that there was decrease in color contributing small molecules as well as large molecules.

Jain et al. (2002) isolated three bacterial strains from the activated sludge of a distillery waste water plant identified as *Xanthomonas fragairae*, *Bacillus megaterium* and *Bacillus cereus* which were found to remove COD and color from the distillery effluent in the range of 55–68% and 38–58%, respectively. Two bacterial strains *Pseudomonas putida* U and *Aeromonas* sp. were used to bioremediate anaerobically treated distillery spent wash in a two-stage bioreactor. In the first stage, *P. putida* reduced the COD and color by 44.4% and 60%, respectively. The *Aeromonas* sp., in

the second stage, reduced the COD by 44%. Algal bioassay was used to evaluate the quality of the spent wash before and after treatment. The spent wash was eutrophic before the experimental treatment, but, after treatment, it showed poor algal growth (Ghosh et al., 2002).

Ghosh et al. (2004) also isolated bacterial strains capable of degrading recalcitrant compounds from anaerobically digested spent wash from soil of effluent discharge site which were identified as *Pseudomonas, Enterobacter*, *Stenotrophomonas*, *Aeromonas*, *Acinetobacter* and *Klebsiella* all of which could carry out degradation of PMDE and maximum 44% COD reduction was achieved using these bacterial strains either singly or collectively.

Chaturvedi et al. (2006) isolated and characterized fifteen culturable rhizosphere bacteria of *Phragmites australis* growing in distillery effluent contaminated sites. These fifteen strains were *Microbacterium hydrocarbonoxydans*, *Achromobacter xylosoxidans*, *Bacillus subtilis*, *Bacillus megaterium*, *Bacillus anthracis*, *Bacillus licheniformis*, *Achromobacter xylosoxidans*, *Achromobacter* sp., *Bacillus thuringiensis*, *Bacillus licheniformis*, *Bacillus subtilis*, *Staphylococcus epidermidis*, *Pseudomonas migulae*, *Alcaligens faecalis* and *Bacillus cereus* which collectively brought about 76% decolorization and 85–86% BOD and COD reduction of the effluent within 30 days

Typically, the bacterial decolorization may require a mixed culture to decolorize molasses wastewater through combined metabolic mode of individual bacterial strains. Thus, mixed culture studies have been carried out by several researchers for degradation of different effluents such as textile effluents. As the catabolic activities of microorganisms in a mixed consortium complement each other, obviously the syntrophic interactions present in mixed communities lead to complete mineralization of the effluent (Alkane et al., 2006; Kumar and Chandra, 2006).

Alkane and his co-workers (2006) reported that 69 % decolorization of molasses spent wash was achieved by using soil samples as inoculum instead of isolated microorganisms. Also, Kumar and Chandra (2006) reported that the additional of 1% glucose as a supplementary carbon source was necessary for molasses decolorization by *Bacillus thuringiensis*, *Bacillus brevis*, and *Bacillus* sp. up to 22%, 27.4%, and 27.4% color removal, respectively. The similar pattern was also observed on the decolorization activity of bacterial consortium DMC, comprising of *Pseudomonas aeruginosa* PAO1, *Stenotrophomonas maltophila* and *Proteus mirabilis*, which achieved its maximum molasses decolorization (67%) and 51% COD reduction within 72 h in the presence of 0.5% glucose (Mohana et al., 2007). Hence,

mixed culture studies seem to be more promising for molasses wastewater decolorization.

2.6.3 Membrane bioreactors

A membrane bioreactor (MBR) combines the biological degradation of waste compounds and the physical separation of the biomass and treated water by membrane filtration. MBRs have been introduced over 30 years ago, and until now one of the large industrial applications have been for wastewater treatment e.g., industrial, domestic and municipal (Yang et al., 2006). They have proven quite efficiency in removing both organic and inorganic contaminants as well as biological compounds from wastewater. MBR associate a suspended growth bioreactor and a filtration through porous membrane, which leads to the total retention of biomass (high microbial concentration) and improved biological reactor operation (Lee et al., 2003). Such systems are most often used as replacement for sedimentation i.e., for separation of biomass. Membranes can also be coupled with bioprocesses for wastewater treatment in two ways. Firstly, they can be used to control the transfer of nutrients into bioreactor or to extract pollutants from wastewaters which are untreatable by conventional biological processes i.e., melanoidins. The target pollutants are then removed in a reactor with the suitable environmental conditions for biological treatment. Secondly, they can be used for mass transfer of gases, usually oxygen for aerobic processes (Brindle and Stephenson, 1996).

According to these positive aspects, MBR has been applied to various wastewater treatments and has successfully treated effluents from a range of industrial wastewaters, including textiles, dairy, food, beverage, paper and pulp, metal fabrication, rendering and chemical manufacture. During the last years the application of membrane bioreactors (MBR) to domestic wastewater treatments has noticeably increased (Judd, 2006) and the interest to apply this emerging technology to industrial wastewater has been growing up (Figure 8).

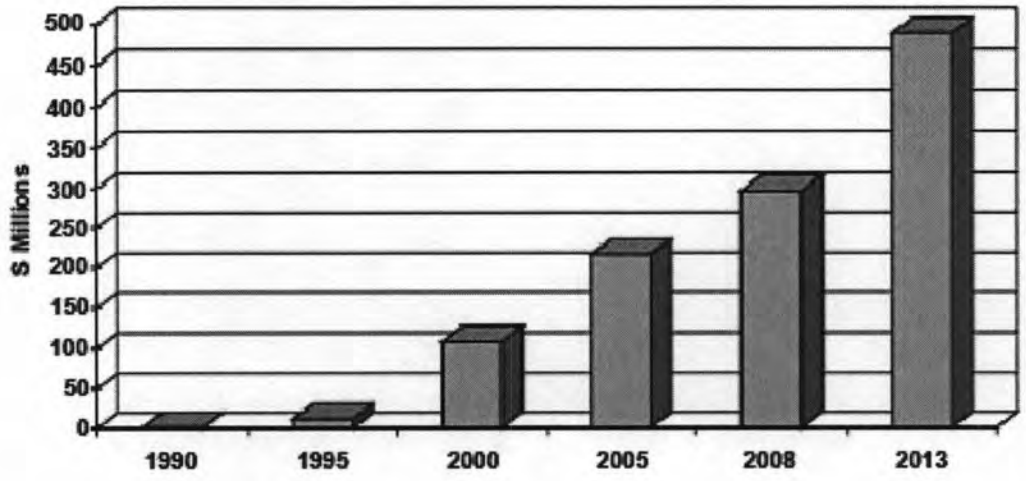

Figure 8 Forecasting the global market value of membrane bioreactor, 1990-2013 (Source: BCC Research)

2.6.3.1 Advantages of membrane bioreactors

There are many advantages in using a MBR process, including superior organics removal, enhanced nutrient removal stability, lower sludge production, smaller footprint, effluent disinfection and high loading rate capabilities (Stephenson et al., 2000). The list of the principle advantages and disadvantages of MBR are shown in Table 6.

Table 6. Advantages and disadvantages of membrane bioreactor.

Advantages	Disadvantages
complete removal of the suspended solids	susceptible to membrane fouling
compact plant size	high capital cost
high rate of degradation	unproven at full-scale, depending on the applications
flexibility in operation	process complexity
low rate of sludge production	
disinfection and odor control	
prolonged microorganisms retention time	
treatment of recalcitrant and toxic pollutants	

2.6.3.2 System configurations

The wide array of membrane types allow MBRs to exist into two major groups according to their configurations; submerged and recirculated:

In the submerged arrangement, the membrane separation unit is submerged in the bioreactor tank. The driving force across the membrane is achieved a suction pump on the permeate line. Aeration and mixing are also achieved by the same unit. Biological degradation occurs in the mixed liquor around the membrane keeping all of the biomass within the reactor (Figure 9). Plate and frame or hollow fiber membranes are available of this configuration.

In the recirculated or external MBR, also called side-stream, the membrane separation unit is outside the main reactor. The biomass is separated externally, and returned to the reactor. A scheme of the recirculated MBR is presented in Figure 10. A high-flow recirculation pump is required for external configuration, the retentate is flowing through a tubular membrane or in-between flat sheet membranes in a cassette. Thus the power requirement is much higher for these membranes than in submerged systems

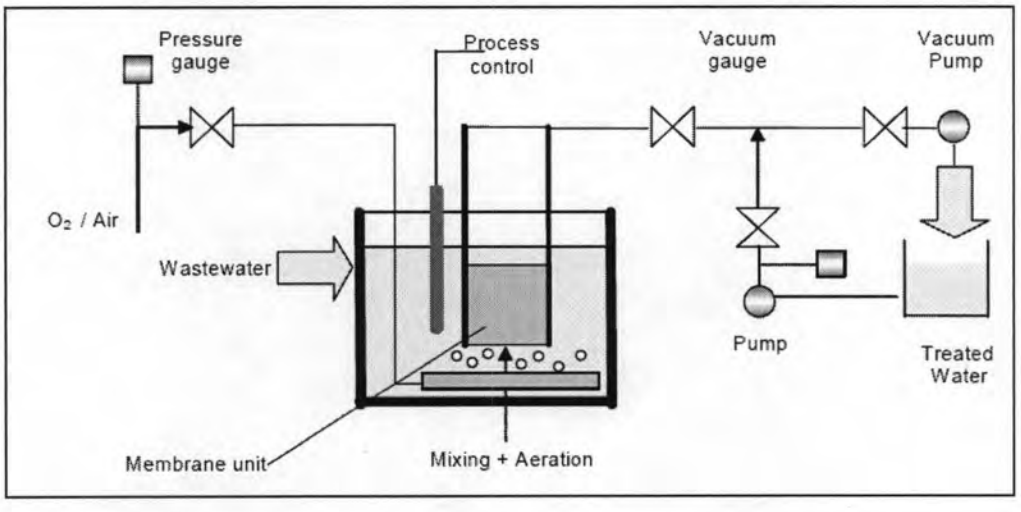

Figure 9 Submerged MBR Configuration - the membrane is situated inside the reaction vessel (Stephenson et al., 2000).

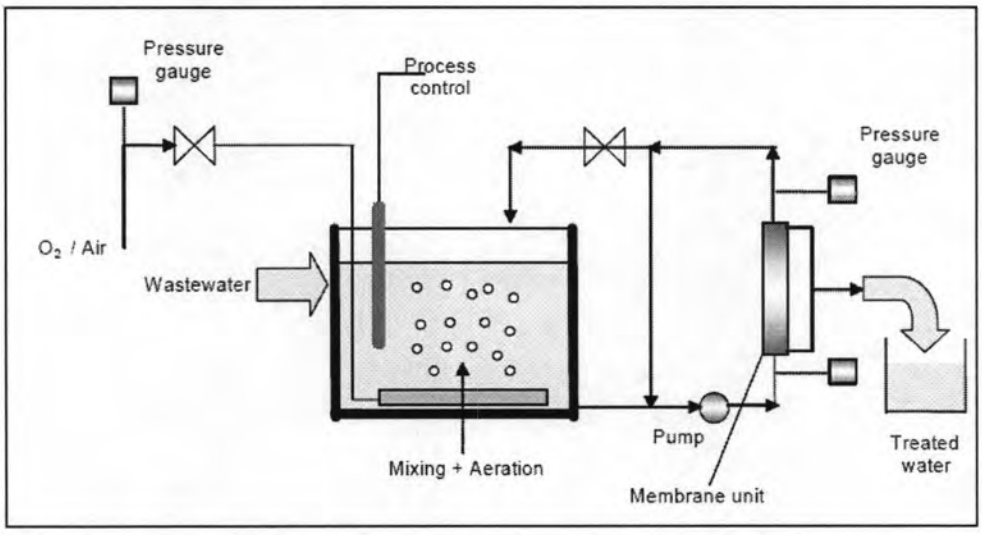

Figure 10 Recirculated or external MBR Configuration: the external MBR, the membrane is placed outside of the main reaction vessel (Stephenson et al., 2000).

2.6.3.3 Membrane materials and configurations

Membrane materials vary widely both in chemical composition and physical structure, but the most fundamentally important property is the mechanism by which separation is actually achieved. A more convenient practical categorization of membranes is according to the material composition, which is generally either organic (polymeric) of inorganic (mineral /ceramic and metallic). The physical structure of the membrane based on these materials can then vary according the exact nature of the material and/or the way in which it is processed. Examples of membrane materials are listed in Tables 7 and 8.

Table 7 Membrane materials by type (Stephenson et al., 2000).

Membrane	Structure	Applications
Ceramic	0.1-10 μm pores	Microfiltration, gas separation, separation of isotopes
Etched polymers	0.5-10 μm cylindrical pores	Analytical and medical chemistry, sterile filtration
Supported liquid	Liquid-filled porous matrix	Gas separation, carrier-mediated transport
Symmetric microporous	0.05-5 μm pores	Sterile filtration, dialysis, membrane distillation
Integral asymmetric microporous	1-10 μm pores at membrane surface	Ultrafiltration, nanofiltration, gas separation, pervaporation
Composite asymmetric microporous	1-5 μm pores at membrane surface	Ultrafiltration, nanofiltration, gas separation, pervaporation
Ion exchange	Matrix of positive and negative charges	Electrodialysis

Table 8 Membrane materials by name (Stephenson et al., 2000).

Materials	Advantage	Disadvantage
Titanium dioxide (TiO_2)	Good thermal resistance	Very expensive
Zirconium dioxide (ZrO_2)	Good Chemical resistance Good mechanical resistance	Limited to microfiltration and ultrafiltration Brittle materials
Cellulose acetate	Inexpensive Chlorine resistant Solvent cast	Poor thermal stability Poor chemical stability Poor mechanical stability
Polysulfone	Steam sterilizable pH resistant Solvent cast	Poor resistance to hydrocarbons
Polypropylene	Chemically resistant	Hydrophobic unless surface treated
Polytetrafluoroethylene (PTFE)	Very hydrophobic Excellent organic resistance Excellent chemical stability Sterilizable	Very hydrophobic Expensive
Polyamide	Good Chemical resistance Good thermal resistance	Sensitive to chlorine

Several types and configurations of membranes have been used for MBR applications. These include tubular, plate and frame, rotary disk, hollow fibers, organic (polyethylene, polyethersulfone, polysulfone, polyolefin, etc.), metallic, and inorganic (ceramic) microfiltration and ultra-filtration membranes. The pore size of membranes used ranged from 0.01 to 0.4 μm. The geometry of the membrane, i.e. in the way it is shaped, is crucial in determining the overall process performance. There are many principal configurations currently employed in membrane processes which all have various practical benefits and limitations (Table 9).

Table 9 Advantages and disadvantages of current membrane configurations (Stephenson et al., 2000)

Configuration	Area/volume Ration (m^2/m^3)	Turbulence Promotion	Advantages	Disadvantages	Applications (most important first)
Pleated cartridge	800-1,000	Very poor	robust construction compact design	Easily fouled cannot be cleaned	Dead end microfiltration
Plate-and-frame	400-600	Fair	can be dismantled for cleaning	Complicated design cannot be back-flushed	Electrodialysis, Ultrafiltration, Reverse osmosis
Spiral-wound	800-1,000	Poor	low energy cost robust and compact	Not easily cleaned cannot black-flush	Reverse osmosis Ultrafiltration
Tubular	20-30	Very Good	easily mechanically cleaned tolerates high TSS waters	High capital and membrane Replacement cost	Cross-flow filtration High TSS waters
Hollow fiber	5,000- 40,000	Very poor	can be back-flushed compact design tolerates high colloid levels	Sensitive to pressure shock	Ultrafiltration, Reverse osmosis

The hollow fibers are used in reverse osmosis to microfiltration, where the water flows from outside to inside the tubes, as well as from inside to outside especially in drinking water processes

The membranes primarily used in wastewater treatment are as follows

Plate and Frame – The plate and frame membranes consist of several flat sheets of membrane material, usually an organic polymer, stretched across a thin frame. The space between the membrane sheets is placed under vacuum in order to provide the driving force for filtration. Several plates are arranged in a cassette to allow to increase the filtration area in a convenient modular design. The membrane cassette is immersed in the mixed liquor and the separation flow is from outside-in.

Hollow fiber – Hollow fiber membranes consist of long bundles, or fibers, of hollow extruded membrane. They are most often of organic polymer. The fibers are potted in a supporting structure that serves as a manifold for permeate transport as well as an air delivery system. Similar to the plate and frame modules, air induced liquid crossflow prevents excessive cake formation and increases the lifespan of the membrane. The selectivity of the hollow fibres are often given by a very thin skin, smaller than 1 micron, which is deposited outside or inside the hollow fiber depending on the permeation mode out/in or in/out respectively. Some of them, called "double skin" membranes have a selective skin on the both sides.

Tubular –Tubular membranes are tubes. Below the membrane surface is a supporting structure with high porosity. In most cases, tubular membranes are made of inorganic material such as ceramic and have a metal oxide membrane surface to provide a small nominal pore size. A tubular membrane could be used in the inside-out arrangement with the feed water flowing along the center of the tube and the permeate passing to the outside walls, or the outside-in arrangement where the influent travels along the outside of the tube and travels axially inside.

2.6.3.4 Permeate flux and crossflow

The key elements of any membrane process are the influence of the following parameters on the overall permeate flux: the membrane resistance, the operational driving force per unit membrane area, the hydrodynamic conditions at the membrane/liquid interface, and the fouling of membrane surface. The flux is the amount of solvent and some components passing through a unit area of membrane per unit time. The flux is determined by both the driving force and the total resistance offered by the membrane and the interfacial region adjacent to it. The resistance of the membrane is fixed, unless it becomes partly clogged by components in the feed water.

In most membrane processes, there are three streams; a feed, a retentate and a permeate streams. The retentate stream is unpermeated products. If there is no retentate stream then operation is termed dead-end (Figure 11 top). Such operation is normally restricted to either low-solids water, such as cartridge filtration of boiler feed water. The alternative to dead-end operation is crossflow operation (Figure 11 bottom), in which the feed-water flows parallel to the membrane surface and so expediting the removal of accumulated material from the membrane. Crossflow operation then implies the existence of retentate stream. The more selective of membrane permeation, and larger the hydraulic resistance, the greater the propensity for crossflow rather than dead end operation.

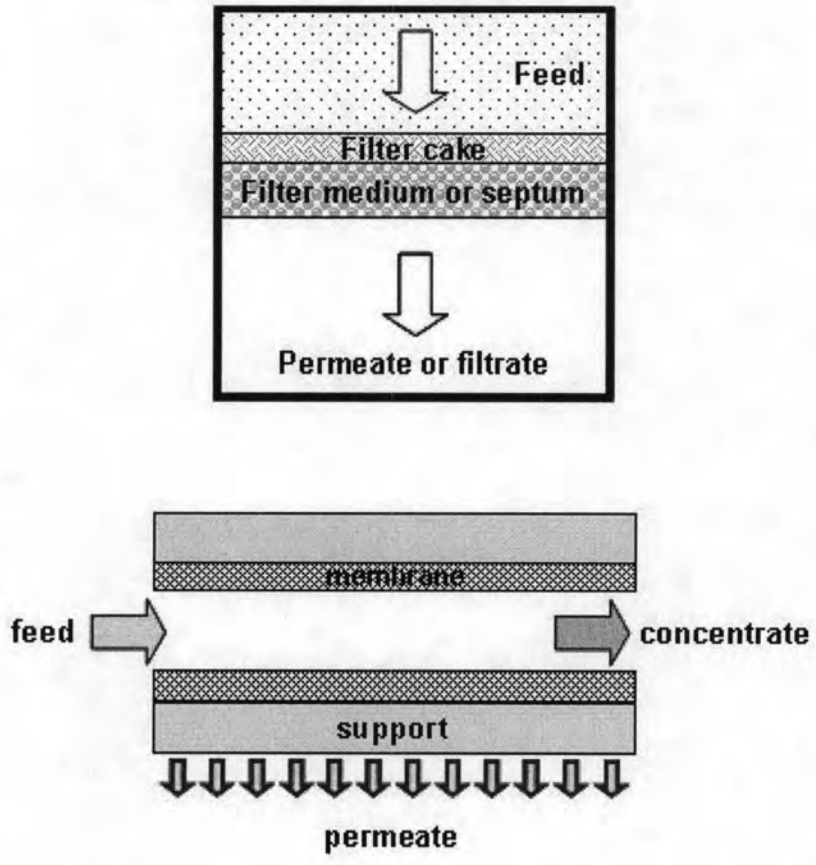

Figure 11 Dead-end (top) and cross flow microfiltration (bottom) (Stephenson et al., 2000).

2.6.3.5 Application of MBR for wastewater treatments

Recently, the membrane bioreactors (MBRs) has proved to be an attractive process for the treatment of industrial and municipal wastewaters as it prevents the loss of biomass from the digester and produces a more constant and better quality of final effluent (Fan and Huang, 2002; Fuchs et al., 2005; Pant and Adholeya, 2007; Satyawali and Balakrishnan, 2008). However, there are very few investigations on distillery wastewater treatment in an MBR. Some of these studies are descried in details as follow.

Choo and Lee (1996) examined a thermophilic (55^{O}C) membrane-coupled anaerobic bioreactor (MCAB) system using 20 kD polymeric plate and frame membrane modules in the external (recirculation) mode. This system was designed for the treatment of alcohol-distillery wastewater during a longtime operation. Enhanced COD removal was achieved with the complete retention of biomass either inside the anaerobic reactor or on the membrane surface. Membrane fouling was mainly attributed to external fouling, which was closely related to the movement of cells to the membrane surface and inorganic precipitation at the membrane surface

Membrane coupled anaerobic bioreactor using 0.2 μm polypropylene and 0.14 μm zirconia skinned inorganic tubular membranes has also been investigated for the treatment of 40,000 mg/l COD distillery wastewater at 55^{O}C. High COD removal (90%) was observed in both the anaerobic MBRs (Kang et al., 2002). In addition, inorganic membrane was found to have accumulated inside the membrane pore and played a key role in flux decline. For the organics, however, a thick cake layer composed of biomass formed on the membrane surface, thus causing a major hydraulic resistance.

Zhang et al. (2006) reported aerobic treatment of simulated distillery wastewater (10,000 mg/l COD) at 30-45^{O}C using 0.2 μm stainless steel membranes. With a HRT of 10–30 h and a VLR of 0.6–2.8 kgCOD·m^{-3}·h^{-1}, mean COD and TN removal efficiencies were 94.7% and 84.4%, respectively.

The reported COD removal efficiencies with mesh/cloth-based MBRs are usually high, but the studies are limited to low influent COD (<2000 mg/l). Fan and Huang (2002) reported 84% COD removal efficiency for a mesh filter MBR operating on municipal wastewater (97.9–371.7 mg/l COD). Similarly COD removal (92%) was observed with a nylon mesh-based MBR operating on municipal wastewater having a COD of 270–570 mg/l (Fuchs et al., 2005). Over 95% COD removal efficiency was observed for food processing wastewater (800 –1,800 mg/l COD) in an MBR

equipped with non-woven fabric having 20 μm pore size (Chang et al., 2007). Furthermore, the BOD:COD ratio of this effluent is relatively low (Satyawali, M. Balakrishnan (2008) due to the presence of recalcitrant organics and growth inhibiting substances (Pant and Adholeya, 2007).

Satyawali and Balakrishnan (2008) investigated operation of a laboratory scale membrane bioreactor (MBR) in the continuous mode for distillery wastewater treatment using submerged 30 μm nylon mesh filters. The study involved acclimatization of municipal activated sludge in a fed-batch reactor followed by operation in a continuous mode at organic loading rates ranging from 3 to 5.71 kg /m^3/day. Up to 41% COD removal was obtained over 245 days of reactor operation, however high molecular weight compounds comprising the color imparting melanoidins remained unaffected. Up to 100% suspended solid retention was obtained and the system could be operated up to 2 weeks without significant flux drop.