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REVIEW

Environmental Hazards of Melanoidin - A Major Colourant in the Distillery Spent Wash - A Review

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Abstract

Distillery spent wash is a very complex, caramelized and cumbersome recalcitrant agro- industrial waste with a temperature range of 70-80^{0}C at discharge point and has deep black brown colour, low pH , high concentration of organic materials and solids. The effluent is recalcitrant due to the presence of melanoidins which are dark black brown colored natural condensation products of sugars and amino acids formed by the non enzymatic browning reactions called Maillard reactions. The distillery effluent is characterized by high biochemical oxygen demand (BOD), chemical oxygen demand (COD), phenol compounds, sulphate and heavy metals. Today the most serious pollution threat caused by melanoidin to the environment is eutrophication in natural water bodies, reduction of sunlight penetration leading to decreased photosynthetic activity, dissolved oxygen concentration in lakes, rivers, lagoons etc. Nevertheless melanoidin pollution on land leads to reduction in soil alkalinity, inhibition of seed germination etc.

Keywords: Distillery spent wash, Melanoidins, Maillard reaction products, BOD, COD

Introduction

Today industries are the major source of pollutants to the ecosystem. Techniques such as distillation and fermentation were known to human civilization since ages. Distilling industries are those concerned with the production of ethanol and distilled spirits such as rum, whisky, brandy, gin and cordials and liquors. Now a days alcohol distillery has emerged as a prominent sector world wide due to the large-scale industrial applications of alcohol in pharmaceuticals, food, perfumery etc. Moreover alcohol is used as an alternate fuel. There are more than 319 distilleries producing 3.25 x 10^9 l of alcohol and generating 40.4 x 10^{10} l of wastewater annually in India alone (Pant and Adholeya, 2007). Apart from other industries distillery is one of the industries which produces wastes characterized by high organic matter, disagreeable colour and odour. In fact alcohol distilleries are listed at the top in the " Red Category" industries as per the Ministry of Environment and Forests (MoEF) due to their high polluting potential (Tewari et al., 2007).

The characteristics of the distillery spent wash are highly dependent upon the raw materials used and the various aspects of the ethanol production processes (Pant and Adholeya, 2007, Satyawali and Balakrishnan, 2008). Distillery spent wash has very high biological oxygen demand (40,000-50,000 mg/l), chemical oxygen demand (80,000-100,000 mg/l) and high BOD/ COD ratio. The amount of inorganic substances such as nitrogen, potassium, phosphates, calcium and sulphates is also very high (Table 1). The recalcitrance of the effluent is due to the presence of melanoidin polymers formed by the non enzymatic browning reactions called Maillard reactions (Plavsic et al., 2006). Melanoidins have antioxidant properties, which render them toxic to many microorganisms such as those typically present in wastewater treatment processes (Kumar et al., 1997a). Melanoidin degradation defiance is due to its escape from various stages of wastewater treatment plants and thus finally entering into the environment. Apart from melanoidins, the other recalcitrant compounds present in the waste are caramel, variety of sugar decomposition products, anthocyanins, tannins and different xenobiotic compounds (Pandey et al., 2003).The presence of skatole, indole and other sulphur compounds those that are not effectively decomposed by yeast during distillation, imparts an unpleasant odour to the effluent (Sharma et al., 2007). The disposal of spent wash into the environment is hazardous and holds 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., 1997a). Melanoidin affects the aquatic life by blocking sunlight penetration in rivers, lakes or lagoons leading to eutrophication (Kumar et al., 1995).

Disposal of distillery spent wash on land is equally hazardous to vegetation. It reduces soil alkalinity and manganese availability thus inhibiting seed germination (Kumar et al. 1997 a). Protein and carbohydrate leaching from the seeds as well as decrease in activities of important enzymes like alkaline phosphatase and ATPase also were observed.

Thus application of distillery effluent to soil without proper monitoring ,seriously affects the ground water quality by altering its physico-chemical properties such as odour, pH , electrical conductivity (EC) etc. due to the leaching down of the organic and inorganic ions (Jain et al., 2005) .

In a study carried out by Dhembare and Amin (Dhemba-

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re and Amin (Dhembare and Amin, 2002) indices indicating soil quality like Sodium Absorption Ratio (SAR), Soluble Sodium Percentage (SSP) and Kelly's ratio were reported to be adversely affected in the soil amended with distillery effluent. Thus the constant disposal / irrigation of the soil with the effluent would definitely lead to a deleterious effect on soil properties. Soil microorganisms are an essential component of the soil ecosystem which is involved in regulating the various processes of nutrient recycling in soil. Thus interfering with their activity may directly affect the soil productivity since they are the indices of soil fertility. Juwarkar and Dutta (1990) evaluated the impact of application of distillery effluent on soil micro flora. Moreover, irrigation with raw distillery effluent resulted in low overall bacterial and actinomycetes count. Nitrogen fixing bacteria Rhizobium and Acetobacter also were found to be reduced considerably.

Table 1. Shows the characteristics of untreated and aaerobically treated distillery effluent. (Mohana,S. Desai, C. and Madamwar,D., 2007; Acharya,B.K., Mohana,S. and Madamwar,D., 2008)

Parameters	Values of distillery effluent	Value of anaerobically treated effluent
pH.5	3.0 – 4.5	7.5 – 8
BOD_5 (mg L^{-1})	50,000 – 60,000	8000 – 10,000
COD (mg L^{-1})	110,000 – 190,000	45,000 – 52,000
Total solid (TS) (mg L^{-1})	110,000 – 190,000	70,000 – 75,000
Total volatile solid (TVS) (mg L^{-1})	80,000 – 120,000	68,000 – 70,000
Total suspended solid (TSS) (mg L^{-1})	13,000 – 15,000	38,000 – 42,000
Total dissolved solids (TDS) (mg L^{-1})	90,000 – 150,000	30,000 – 32,000
Chlorides (mg L^{-1})	8000 – 8500	7000 – 9000
Phenols (mg L^{-1})	8000 – 10,000	7000 – 8000
Sulphate (mg L^{-1})	7500 – 9000	3000 – 5000
Phosphate (mg L^{-1})	2500 – 2700	1500 – 1700
Total nitrogen (mg L^{-1})	5000 – 7000	4000 – 4200

Melanoidin Formation Pathway

In general melanoidins are natural condensation products of sugar and amino acids produced by Maillard reactions, which is one of the major reactions taking place during the thermal processing, household cooking and storage of foods (Painter, 1998). In foods, the amino compounds mostly exist in free or protein bound state and the reducing compounds as reducing sugars. Maillard (1912) was the first to observe the darkening, following the reaction of sugars with amino acids. The formation of brown pigments and polymers during the reaction of the amino groups of amino acids with the carbonyl groups of sugars were demonstrated by Maillard in 1916. Since then, the browning reaction between sugar and amino compounds is commonly called as non-enzymatic Maillard reaction (Fig.1) producing a number of compounds.

Thus melanoidin formation 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 Maillard reaction complexity has been studied extensively during recent years and new pathways and key intermediates have been established (Martins et al., 2001). A scheme of Maillard reaction is shown in Figure 2.

Melanoidins are considered to be acidic compounds with charged nature. With reaction time and temperature, the total carbon content also increases, which promotes unsaturation of the molecules. Thus the color intensity increases with the polymerization degree.

The formation of a C3 sugar fragment in early stages of browning reaction between sugar and amines or amino acids, identified as methylglyoxal dialkylamine was reported by Hayase et al., (1982). Fay and Brevard (2004) 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, and 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. The reaction thus yielded 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 and hence was named as Amadori compounds. Studies on marine humic and fulvic acids revealed that they are formed by the condensation of sugars with amino acids or proteins via Maillard reaction and the main building blocks of humic substances are the heterocyclic moieties rather than aromatic benzenoid structures (Ikan et al., 1992). Hayashi and Namiki (1986) have observed that C3 imine formation followed the pattern of C2 imine formation, and was well correlated to the decrease in the amount of glucosylamine and an increase in the formation of Amadori products.

Reaction of Amadori products with n-butylamine rapidly produced C3 compound in a manner similar to that of glucose-n-butylamine system which indicated the possibility of participation of Amadori products in the formation of C3 compound. In spite of large research work done, mechanism of melanoidins formation at later final stages of Maillard reaction is still obscure

Structure of Melanoidin Polymer

It is very difficult to separate food/natural melanoidin from other food constituents and therefore the chemical and biological studies on melanoidins have been done on model

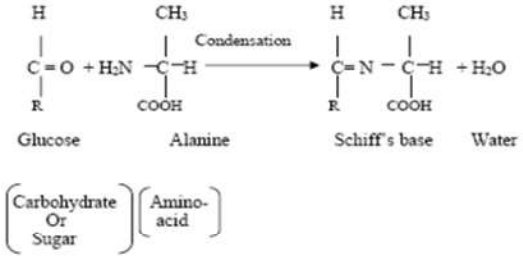

Figure 1. Non- Enzymatic Maillard Reaction

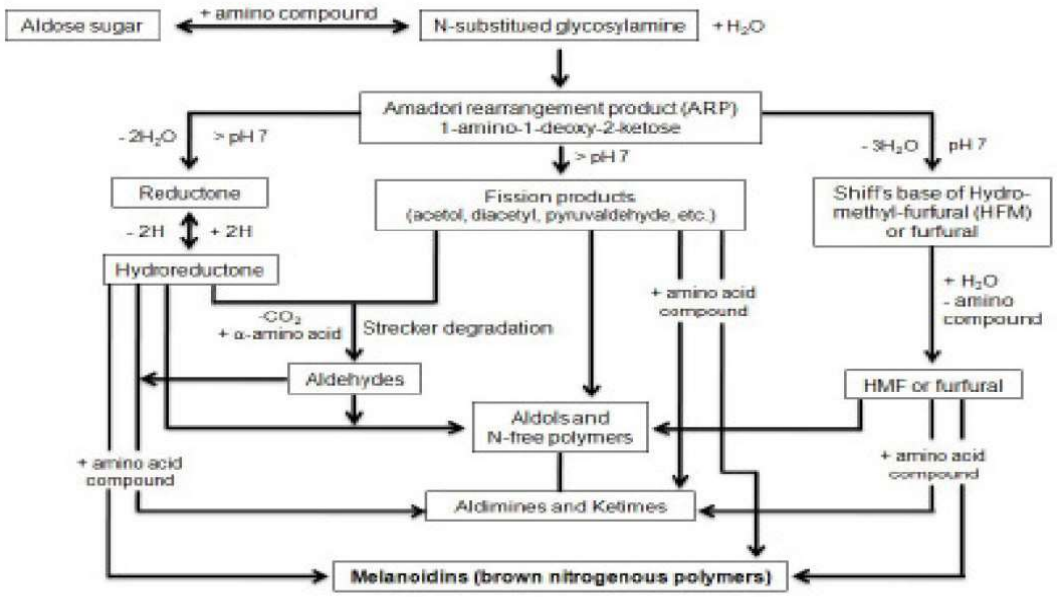

Figure 2. Scheme of Maillard reaction (Martins et al., 2001).

melanoidin. Even though the chemical structure of melanoidin is not yet clear, some part of the chemical structure of model melanoidin has recently been elucidated by different spectral studies such as 1H NMR, CP-MAS NMR etc by various workers (Ikan et al.,1990, 1992; Larter and Douglas, 1980). Chemical investigations of natural and synthetic melanoidins have revealed that both have similar elemental (CHOH) compositions, spectroscopic properties and electrophoresis mobilities at various pH values (Migo et al., 1997; Ikan et al., 1990, 1992). However the nitrogen contents, acidities and electrophoretic behaviour of polymers all reflect the functional group distributions inherited from the amino acids (Hedges, 1978).).Studies on xylose- glycine N15 melanoidin by N15 cross polarized – magnetic angle spinning (CP-MAS) NMR by Benzing et al. (1983) have reported that the nitrogen in melanoidin polymers exists mainly in secondary amide form and some as pyrole and or as indole nitrogen and also revealed that sterically hindered secondary amide bonds are very resistant to acid hydrolysis. According to (Hayase et al., 1984) the melanoidin structure seems to have CH3- COR moiety and C-terminal structures originated from glucose existing in melanoidins are suggested as follows:

1. CH3-CO-R,
2. CH3-C (H or OH) =C (H or OH)-CO-R". R-CO-CO-R'
3. R-CO-CH (CH3)-CO-R'
4. R-CO-CH2-CO-R'
5. R-CO-CH2-CH2-CO-R'
6. CH3-CH (OH)-CO-R and so on.

A general structure for melanoidins prepared from monosaccharide and glycine was proposed by Cammerer and Kroh (1995). The chemical structure of investigated melanoidins mentioned above is shown in Figure 3.

Later on, Cammerr et al., (2002) had again proposed the basic structure of melanoidins formed from carbohydrates and amino acid reaction shown in figure 4.

Due to the net negative charge , heavy metal ions (Cu^{2+} , Cr^{3+} , Fe^{2+}, Zn^{2+}, Pb^{2+} etc.) form large complex molecules with melanoidins, amino acids, proteins and sugars in acidic medium and get precipitated (Migo et al., 1997). According to Hayase et al. (1986) the principal skeleton or backbone of melanoidins are comprised up of saturated and aliphatic carbon atoms. Melanoidin chromophore structure consists of unsaturated bonds which are formed due to the cleavage of C = C or C=N bonds and was speculated that the nitrogen in melanoidin was mainly due to the conjugated enamine linkage and partly to amine linkage.

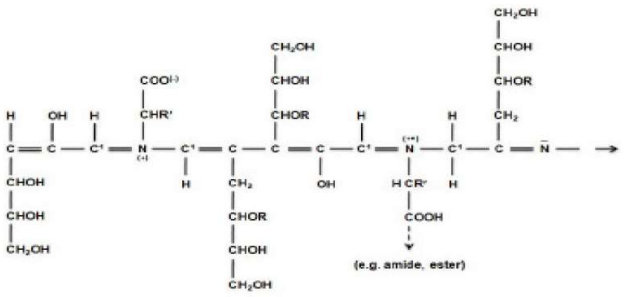

Figure 3. Proposal for the general structure of the melanoidin polymer (Cammerer and Kroh, 1995) R:H or saccharides, R'; side chain of amino acid

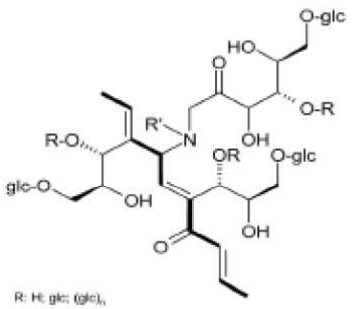

Figure 4. Basic melanoidin structure formed from carbohydrates and amino acid (Cammerer et al., 2002)

Melanoidin chromophore has not been yet properly understood and hence the chemical structure of the so called melanoidins is still unclear. Probably it does not have a definite one since there exist various types of melanoidin differing in structure depending on parent reactants and reaction conditions as pH, temperature and reaction time. There is need for intensive research with most refined, recent and advanced techniques for the elucidation of chromophore structure to deduce the main skeleton of melanoidin polymer.

Behaviour and Reaction of Melanoidins in the Environment

Melanoidins are found in natural waters, river waters, estuarine, coastal and open waters whereby representing a key link in the transformation of labile organic matter (polysaccharides, aminoacids) into more recalcitrant humic material in nature / environment. The similarities of these melanoidins with HA (Humic Acids) make them suitable buffer compounds for metallic ions (Ikan et al., 1992). The extent of melanoidin complexation depends on the aminoacids used to make them and the highest complexing capacity is obtained for melanoidins prepared from glucose and the basic aminoacid lysine (Painter, 1998).

The complexing properties of melanoidins towards the metal ions are influenced by the presence of calcium and magnesium ions and other macro and micro constituents. Melanoidins prepared using condensation times longer than two days exhibit complexation properties towards copper ions that appear to depend on the basicity of the amino acid precursor and the molecular mass of the product (Plavsic et al., 2006). Higher pH value changes the ligand configuration by making more available binding sites. The highest copper ion complexing capacity values were obtained for melanoidin obtained from glucose- lysine with molecular mass > 10 Kda as lysine contains two amino groups while glutamic acid and valine contains only one (Plavsic et al., 2006). Basic amino acids (i.e., those containing more amino groups than carboxylic groups) preferentially condense with sugars to form nitrogen- rich polymers (Hedges, 1978), which are good complexing agents for copper ions.

It is reported that melanoidins behave as anionic hydrophilic polymers which forms stable complexes with metal cations and that ketone or hydroxyl groups of pyranone or pyridone residues act as donor groups in melanoidins and participate in the chelation with metals as melanoidins have net negative charge. Therefore , different heavy metals (Cu2+, Cr3+,Fe3+, Zn2+, Pb2+ etc) form large complex molecules with melanoidins, amino acids, proteins and sugars in acidic medium and get precipitated (Migo et al., 1997).

Studies have revealed that trivalent metal ions form stronger complexes with HA than monovalent and divalent metal ions (Painter, 1998). There were no significant differences between the different melanoidins formed from the Maillard model systems. But widely different behaviour was observed in the ability to bind iron among melanoidins isolated from commercial coffee, sweet wine and beer due to bilinear behaviour, which indicates the presence of at least two different types of binding sites (Morales et al., 2005). Studies have shown that the low molecular weight compounds binding the melanoidins exerts higher antioxidant and antimicrobial activities than those of the pure melanoidins. Melanoidins obtained from His- Glu reaction mixture are elucidated as furan ring and nitrogen containing brown compounds having peroxyl radical scavenging activity. This is an indicator of highest ant oxidative activity determined by conjugated diene formation from per oxidation of linoleic acid (Yilmaz and Toledo, 2005).

The heated sugar – casein model melanoidins consisting of variable sugars exhibit different mutagenic activity was demonstrated by Brands et al. (2000).It has been proved that the ketose sugars (fructose and tagatose) have higher mutagenicity than their aldose isomers (glucose and galactose) and generates active oxygen species resulting in DNA strand breaking and mutagenesis. It has been reported that some MRPs (Maillard Reaction Products) induce chromosome aberrations in Chinese hamster ovary cells and gene conversion in yeast. . Mutagenicity and DNA strand breaking activity of melanoidins from a glucose- glycine model was demonstrated by Hiramoto et al. (1997) who reported that the LMW fractions act as lipid sink (Larter and Douglas, 1980) and induced DNA damage, which increased with increase in concentration. High concentration 1% is cytotoxic for the cells, while lower concentrations 0.5% to 0.2% found to reduce cell proliferation and cell viability.

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