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A Review on the Role of Microbes in Degradation of Melanoidin from Distillery Wastewater

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Abstract— *Discharge of melanoidin containing distillery wastewater raises a serious environmental concern as it can pose severe health risks to aquatic bodies and soil due to absorption of sunlight, change in the alkalinity and inhibition of seed germination. Several physicochemical and biological based clean-up technologies have been investigated to combat this environmental pollution. Strategies to decolorize the distillery effluents using potential microbial communities are efficient and cost-effective. Distillery effluent treatment methods using fungi, algae and bacteria have demonstrated promising results. Microbial enzymes involved in the mechanism of decolorization have also been studied extensively. Current advances in melanoidin decolorization using nanoparticles show great promise for treating industrial effluents. The focus of the present review is to explore the current approaches of use of different groups of microbes and novel approaches such as use of nanoparticles in decolorization of melanoidin containing distillery wastewater.*

Keywords— ***Distillery wastewater, melanoidin, bacteria, fungi, algae, nanoparticles***

I. INTRODUCTION

Distilleries are among the major industries in India, generating significant volumes of wastewater, often referred to as spent wash or raw effluent, which can pose serious risks to soil and water quality. This effluent, produced during ethanol production, is a brown-colored liquid characterized by a high concentration of organic matter and nitrogen compounds, low pH, elevated temperature, and high salinity. The quality of the substrates and the unit activities employed to produce alcohol determine how polluted distillery effluent is, hence, effluent characteristics might vary from distillery to distillery. Molasses is frequently used as a raw material for alcohol manufacturing, due to its high carbohydrate content, along with its abundance of minerals and organic acids, making it an ideal fermentation substrate. The quality and composition of molasses can differ greatly based on factors such as the type of sugarcane, harvesting methods, and processing techniques employed [1,2].

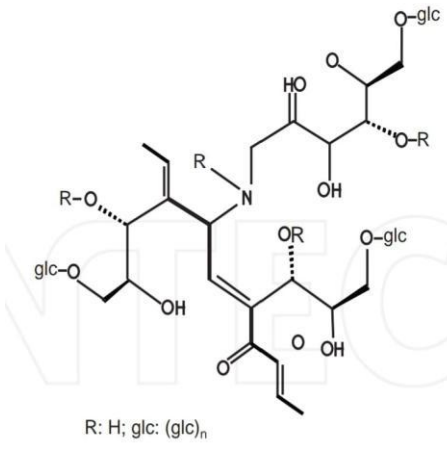

Fig: Basic structure of melanoidin [3]

Molasses based distilleries typically produce 8-15L of effluent, which is distinguished by high chemical oxygen demand (COD) and biochemical oxygen demand (BOD), for every litre of alcohol produced. The effluent's high COD

and BOD levels are caused by the presence of several organic components, including proteins, polysaccharides, polyphenols, waxes and melanoidin [4,5]. Because of this, spent wash needs to be pretreated before it can be properly disposed of in the environment.

Melanoidin emissions from alcohol distilleries present serious environmental problems, especially for aquatic environments. Because of its dark pigmentation, sunlight is absorbed by it, decreasing light penetration in water bodies and preventing aquatic plants and algae from photosynthesizing. The equilibrium of aquatic ecosystems is further impacted by this interruption in photosynthesis, which lowers oxygen production. Furthermore, because microorganisms use a lot of oxygen to break down melanoidin, its high organic content raises the biochemical oxygen demand (BOD) in water. As a result, dissolved oxygen levels are reduced, which frequently leads to anaerobic conditions that are detrimental to fish and other oxygen-dependent aquatic life. Since the effluent is high in nutrients like phosphorous and nitrogen, melanoidin in water also contributes to eutrophication [6]. These nutrients encourage algae blooms, which decompose and consume more oxygen, resulting in hypoxic or "dead zones" that are inhospitable to aquatic life. Additionally, melanoidin contains harmful substances that can directly impact aquatic plant and animal development, reproduction, and survival, hence decreasing biodiversity and upsetting ecosystems. Because of its resistance and persistence, it is difficult to break down and poses a long-term risk to groundwater and surface water. To lessen melanoidin's negative environmental effects and safeguard aquatic ecosystems, efficient wastewater treatment techniques like microbial degradation or improved oxidation processes are crucial.

Physical and chemical techniques such as ozonization, flocculation, chemical coagulation, precipitation, activated carbon adsorption, and advanced oxidation processes are examples of conventional methods for decolorizing melanoidins [7]. The rapid efficacy of these techniques in lowering color and organic load makes them popular. These strategies, however, have significant drawbacks that compromise their viability. For example, chemical processes like ozonization and coagulation can result in the production of enormous amounts of sludge, whereas physical techniques like activated carbon adsorption can be expensive because adsorbents must be replaced frequently. Additional treatment and disposal of this sludge will increase operating expenses and environmental concerns.

II. COMPOSITION OF DWW AND ITS ENVIRONMENTAL EFFECTS

In India, there are more than 325 distilleries that produce around 45 billion litres of wasted wash and 3 billion litres of alcohol yearly [8,9]. The complicated and highly contaminated character of distillery wastewater makes it one of the most difficult industrial effluents to handle. Among its many undesirable characteristics are its high temperature, low pH, dark brown colouring, substantial ash content, and large quantities of dissolved organic and inorganic materials. It also shows extremely high levels of chemical and biochemical oxygen demand (BOD and COD) which is a major environmental issue. The property of this effluent depends on the kind of feedstock and the procedures utilized to produce ethanol. During the anaerobic treatment process, the pH of spent wash rises from 4.5 to 8.5, and it is ultimately referred to as post-methanated distillery effluent (PMDE) [10]. The spent wash is a waste with extremely high levels of nitrogen (2,200 mgL^{-1}), phenolics (4.20 mgL^{-1}), sulphate (3,410 mgL^{-1}), total solids (TS; 82,480 mgL^{-1}), chemical oxygen demand (COD; 90,000-1,10,000 mgL^{-1}), and biochemical oxygen demand (BOD; 35,000-40,000 mgL^{-1}). Several heavy metals, including Cd, Mn, Fe, Zn, Ni, and Pb, are also found in addition to these pollutants. The anaerobic treatment procedure that the wasted wash predominantly goes through transforms a substantial amount (>50%) of the BOD and COD. Anaerobic digestion, however, causes distinct metabolic alterations in the spent wash. The reduction of oxidized sulphur compounds results in the creation of a sizable quantity of hydrogen sulphide (H_2S). Sulphide creates a colloidal solution of metal sulphide colorant by binding with the heavy metals in the effluent [11]. If left untreated, this enormous amount of wastewater, roughly 40 billion litres can put a great deal of strain on the waterways and harm aquatic species on a large scale.

The primary barrier to waste remediation is the color of the distillery spent wash (DS), which contains melanodin pig ment at a weight percentage of about 2% [12]. This dark brown complex biopolymer is formed because of Maillard amino-carbonyl reaction, a non-enzymatic browning process that occurs between the amino and carbonyl groups in organic materials [13,14,15]. The Maillard reaction between D-xylose and glycine produces colorful molecules [16]. These pigments were thought to be crucial intermediates in the production of melanoidins because they became brown when they broke down. A new blue pigment known as BlueM1 has been identified which has a methine proton between two pyrrole rings and are made up of four D-xylose and glycine molecules [17]. Disaccharides and amino groups from amino acids combine to generate a Schiff base in the first step of the Maillard reaction, after

which they undergo transformation via the Amadori rearrangement product [18,19].

In addition to melanoidins, DWW reports the presence of many other harmful substances, including 2-hydroxysocaproic acid, benzene propanoic acid, di-n-octyl phthalate, and di-butyl phthalate [20,21]. These harmful substances, especially phthalates, are known to be endocrine disrupting chemicals (ECDs), which lead to hormonal imbalances and several physiological and metabolic conditions that impair both human and animal reproductive fitness [22,23].

Melanoidins are harmful to agricultural crops, prevent seed germination, and result in a manganese shortage in the soil. Even at low concentrations of 5% (v/v), raw distillery effluent has a severely harmful effect on *Vigna radiata* seed development and germination [24]. Long-term usage of untreated or inadequately treated effluents can alter the pH, increase electrical conductivity (EC), and exchangeable salt levels, among other important soil characteristics. Crop production and soil health are negatively impacted by these alterations, which result in soil salinity and alkalinity. Distillery wastewater's organic content causes organic acids to develop during decomposition, momentarily immobilizing plant nutrients and preventing crops from accessing them. Additionally, microbial diversity is frequently decreased in effluent-irrigated soils, with fungi and actinomycetes increasing and nitrogen-fixing bacteria decreasing. Long-term soil fertility may be impacted by the disruption of nutrient cycles caused by this microbial imbalance. The buildup of salts and harmful substances, such heavy metals like copper, manganese, and zinc, further deteriorates soil quality and endangers crop safety. The possibility of long-term soil damage owing to salt buildup is still a major worry, even though post-methanation distillery effluents have higher pH values and lower organic loads, making them relatively safer for agricultural use. To reduce these negative impacts while utilizing the wastewater's nutritional potential, sustainable management techniques are crucial. These include dilution of effluents, periodic rest intervals for soil recovery, and regulated application rates.

It has been reported that several refractory components found in molasses effluent, such as xenobiotic substances, anthocyanins, caramel, sugar breakdown products, melanoidin, and tannins, are resistant to degradation and remain in the environment. Decomposable organics such skatole, indole, and sulphur compounds are the source of its disagreeable smell. It is extremely hazardous to the environment and has a foul stench when dumped into rivers or canals. since of the large organic and chemical load of the effluent, its untreated disposal poses a major pollution risk since it causes oxygen depletion, water contamination, and damage to aquatic ecosystems. For these effects to be lessened, proper therapy is essential.

III. CURRENT APPROACHES ON THE TREATMENT OF DISTILLERY EFFLUENT

The treatment of distillery effluent aims to eliminate undesirable substances present in the wastewater so that it can be safely released into the environment. Various treatment approaches have been done to reduce the pollution load from the spent wash, which includes treating the wastewater with physio-chemical methods (coagulation and flocculation, electrocoagulation, adsorption, advanced oxidation, membrane treatment), and biological methods (aerobic, anaerobic and enzymatic). Given that the presence of melanoidin is hazardous, efforts have been made to understand its chemical structure and lower its emissions through chemical and microbiological degradation. This will allow for the development of more effective solutions for decolorization and degradation.

3.1 Enzymatic mechanism of DWW decolorization

Numerous enzymes have been identified by various sources as being crucial to waste treatment procedures, including peroxidases, oxidoreductases, cellulolytic enzymes, cyanidase, proteases, amylases, etc [25,26]. The two primary categories of enzymes that make up the ligninolytic system are laccases and peroxidases, which include lignin and manganese peroxidases [27]. Because they oxidize both toxic and non-toxic substrates, bacterial laccases are crucial to the bioremediation of industrial waste. Laccases are a fascinating class of common oxidoreductase enzymes that have a lot of potential for use in biotechnological applications. Numerous studies have proposed the involvement of multiple enzymes with distinct processes in DWW decolorization. Thus, understanding enzymes in the bioremediation of different industrial pollutants will lead to numerous prospects for widespread use [28,29].

3.2 Microbial degradation of melanoidin

Microbial degradation and decolorization of distillery effluents are an economical and environmentally beneficial substitute for physiochemical approaches. Numerous microorganisms, including bacteria, fungus, and algae have been documented for their capacity to degrade and discolourise the distillery effluent. Melanoidin can be eliminated by microorganisms by enzymatic breakdown, flocculation by chemicals released by the microbes, adsorption onto the surface of living (resting) and dead (autoclaved) cells and using the pigment as a source of carbon and nitrogen [30,31]. It has been documented that a variety of intracellular and extracellular enzymes including

laccases, manganese peroxidases, lignin peroxidases and sugar oxidases like sorbose oxidase, exhibit melanoidin degrading activity [32,33].

Microorganisms, particularly white-rot fungi, produce a range of nonspecific extracellular enzymes, including lactase, H_2O_2, and oxidases, including lignin peroxidases and manganese peroxidase (MnP). Using H_2O_2, lignin peroxidase (LiP) oxidatively breaks down lignin. As substrates, lignin peroxidases (LiP) and manganese peroxidases (MnP) oxidize Mn^{2+}, phenolic and non-phenolic compounds, and different hues [34].

The catalytic mechanism of LiP and MnP differs in that the former catalyzes the one-electron oxidation of phenolic and non-phenolic compounds by H_2O_2, which induces the production of the corresponding free radicals, whereas the latter catalyzes the oxidation of Mn(II) to Mn(III) dependent on H_2O_2, after which the oxidized Mn(III) catalyzes the one-electron oxidation of phenolic and non-phenolic compounds by H_2O_2, which also produces the corresponding free radicals.

A wide range of contaminants, including melanoidins, are degraded by the free radicals produced by the microbes. Several fungal, bacterial, and algal species produce H_2O_2, laccase, manganese-dependent peroxidase (MnP), and lignin peroxidase (LiP), including *Bacillus licheniformis*, *Alcaligenes sp., Penicillium pinophilum*, *Alteraria gaisen*, *Coriolus hirsutus*, *Emericella nidulans*, *Flavodon lavus*, *Oscillatoria boryana* (BDU 92181) and *Neurospora intermedia*[35,36,37,38]. Thermophilic cutinase from *Thermobifida alba* demonstrated the best decolorization efficacy and removed 76.1-78.2% of colorants. *Thermobifida alba* cutinase was immobilized on a modified chitosan carrier that was both economical and effective, and it produced a decolorization yield of 79.3–81.2% [39].

3.2.1 Fungal degradation

Numerous fungal species, including *Aspergillus fumigatus* G-2-6, *Emericella nidulans var. lata, Geotrichum candidum, Trametes sp., Aspergillus niger, Citeromyces sp., Flavodon flavus*, and others, have been employed by different providers to treat DWW [40,41,42,43].

In addition to producing some important byproducts like protein-rich fungal biomass that can be utilized as animal feed or other fungal metabolites, fungal treatment is used to lower COD, BOD, and the breakdown of organic compounds. Filamentous fungi have less nucleic acid in their biomass and are less sensitive to changes in temperature, pH, nutrients, and aeration [44].

Under ideal conditions—5 g/L of fructose, 3 g/L of peptone, 5 pH, and 35˚C-*Cladosporium cladosporioides* was able to minimize 52.6% color and 62.5% chemical oxygen demand from DWW [45]. *Cladosporium cladosporioides* was also employed under various settings, including fructose concentration 7 g/L, peptone concentration 2 g L, pH 6, and 10% (w/v) inoculum concentration, where a decrease of 62.5% and 73.6% in color and COD were observed, respectively [46]. Furthermore, *Aspergillus niger* was used in conjunction with combined coagulants to demonstrate a 97.2% color reduction from DWW [47]. Some white rot fungi are also known to secrete ligninolytic enzymes (LiP, MnP, and Laccases) that can break down xenobiotics and organometallic contaminants. Some yeast strains such as *Candida glabrata, C. tropicalis* and various fungi such as *Aspergillus niger* and white rot fungi such as *Phanerochaete chrysosporium* have been reported to effectively decolourise melanoidin from distillery effluents [48,49,50].

3.2.2 Algal degradation

Researchers are interested in treating DWW using microalgae because of the waste's products and byproducts, which are highly sought after for social welfare [51]. The potential of DWW bioremediation was examined in conjunction with a novel *Chlorella sorokiniana* sp. grown in a high-density photo bioreactor in a semi-batch mode. Since the process is energy efficient and can meet its nutrient requirements from biomethanated spentwash and energy requirements from sunlight, micro algal treatment only becomes effective after the anaerobic treatment of spent wash [52].

A 52% color decrease was achieved when 10% DWW was anaerobically treated with the microalgae *Chlorella vulgaris* and *Lemna minuscula* [53]. Additionally, a marine cyanobacterium called *Oscillatoria boryana* (BDU 92181) was also studied that degraded 5% melanoidin. *Oscillatoria willei* also exhibited increased oxidative stress and a rise in ligninolytic and anti-oxidative enzymes like lignin peroxidase, laccase, polyphenol oxidase, superoxide dismutase, catalase, peroxidase, and ascorbate peroxidase when grown with a lower nitrogen content but with optimal phenolic compounds. According to the study's findings, the Cyanobacterium *O. willei* decolorized the substrate phenol by up to 52% in just seven days thanks to these enzymes. Given the size of the wastewater market, combining the production of microalgae biomass with nutrient removal/pollutant degradation may thus signify a significant turning point in the bioenergy ambitions.

3.2.3 Bacterial degradation

An inexpensive and environmentally beneficial substitute for physico-chemical wastewater treatment methods is the bacterial breakdown and decolorization of industrial wastewaters. Many studies have recently employed pure culture and bacterial consortiums to effectively decolorize and degrade DWW. According to DWW, the bacterial

consortium made up of *Pseudomonas aeruginosa* PAO1, *Stenotrophomonas matophila*, and *Proteus mirabilis* reduced color and COD by 67% and 51%, respectively, in 24 and 72 hours at 37˚C, it was reported utilizing a combination of *Klebsiella oxytoca, Serratia marcescens*, and *Citrobacter sp*. to remove color from Viandox sauce (13.5% v/v), caramel (30% w/v), beet molasses wastewater (41% v/v), and sugarcane molasses wastewater (20% v/v) in 9.5, 1.13, 8.02, and 17.5% of cases in just two days [54]. Additionally, in 48 hours under aerobic conditions, they used a consortium of *Acinetobacter sp., Pseudomonas sp., Comamonas sp., Klebsiella oxytoca, Serratia marcescens,* and an unidentified bacterium to remove 26.5% of the color from DWW [55].

The capacity of bacterial cultures to break down these pigments was assessed. The melanoidins were recently decolorise using lactic acid bacteria (*Lactobacillus coryniformis, L. sakei, L. plantarum, Weisella soli, Pediococcus parvulus,* and *P. pentosaceus*). 44% of melanoidins are decouraged by the strain *Lactobacillus plantarum* [56]. An attempt was made to decolorize the melanodins by employing *Bacilli* consortia where the capacity to remove color was evaluated in two mixed bacterial cultures (C1 and C2) of the species *Bacillus* [57]. The consortiums *Proteus mirabilis* (IITRM5; FJ581028), *Bacillus sp.* (IITRM7; FJ581030), *Roullella planticola* (IITRM15; GU329705), and *Enterobacter sakazaki*i (IITRM16, FJ581031) were also created in a 4:3:2:1 ratio. Within ten days, this consortium oversaw 75% of the melanoidins' decolorization [58].

At the ideal pH of 7.5 and temperature of 37 °C, the isolate *Alcaligenes faecalis* strain SAG5 exhibited 72.6% melanoidin decolorization on the fifth day of incubation. The mung bean (*Vigna radiata*) toxicity study showed that the raw distillery effluent was extremely harmful to the environment in comparison to the biologically treated distillery effluent, indicating that the effluent following bacterial treatment is safe for the environment [59].

Many different bacterial cultures, including *Pseudomonas putida, P. aeruginosa, Lactobacillus plantarum, Bacillus circulans, B. megaterium, B. irmus, B. thuringiensis, B. cereus, Lactobacillus hilgardii, L. coryniformis,* and *Xanthomonas fragariae,* have been shown to be active in decolorization and degradation of distillery effluents [60,61,62,63].

From soil contaminated with distillery effluent, a thermotolerant bacterial culture consisting of *Bacillus subtilis, B. cereus*, and *Pseudomonas aeruginosa*. Of them, *B. subtilis* demonstrated the greatest degree of decolorization (85%) at 45°C using relatively low carbon (0.1%, w/v) and nitrogen sources (0.1%, w/v) throughout the course of a brief 24-hour incubation period [64]. Under ideal circumstances*, Pseudomonas sp*. and *B. cereus* had decolorization rates of 69% and 73%, respectively. *B. subtili*s demonstrated the strongest thermotolerance, withstanding temperatures between 35°C and 50°C without affecting the exponential development phase. The genus *Bacillus* demonstrated the best bioremediation efficiency when compared to other bacterial cultures, according to findings from several examinations.

Three thermotolerant bacterial isolates- *Bacillus nitratireducens* (B2), *B. paramycoides* (B1), and *Brucella tritici* (B3) were shown to be melanoidin-decolorizing agents in research. These isolates were further optimized for decolorization under a range of nutritional and physicochemical conditions. After 40 hours of incubation under static circumstances with 0.5% glucose (w/v), 0.5% peptone (w/v), 0.05% MgSO4, and 0.01% KH2PO4 at a pH of 6.0, *B. nitratireducens* (B2) showed the greatest degree of decolorization (86%) of the three species at 40°C [65].

Utilizing the axenic and mixed bacterial consortia [*Bacillus licheniformis* (RNBS1), *Bacillus sp.* (RNBS3), and *Alcaligenes sp.* (RNBS4)], the degradation of synthetic and natural melanoidins was investigated. The mixed consortium was more successful than axenic cultures in decolorizing synthetic and natural melanoidins by 73.7% and 69.8%, respectively, while axenic cultures RNBS1, RNBS3, and RNBS4 decolored synthetic melanoidins by 65.88%, 62.5%, and 66.1% and natural melanoidins by 52.6%, 48.9%, and 59.6%, respectively. In comparison to controls, the HPLC analysis of degraded samples revealed fewer peak regions, indicating that the breakdown of melanoidins by isolated bacteria may be primarily responsible for the color intensity drop.

Lactobacillus plantarum (No. PV71-1861) isolated from pickle samples exhibited greatest melanoidin pigment (MP) decolorization yield of 68.12% using an MP solution that included 2% glucose, 0.4% yeast extract, 0.1% KH2PO4, 0.05% MgSO47H2O, an initial pH of 6 and in a 7-day static condition at 30 °C [66].

Bacterial consortium containing three bacterial cultures showed the ability to decolorize and degrade wastewater quickly demonstrating 67 ± 2% decolorization in 24 hours and 51 ± 2% reduction in chemical oxygen demand in 72 hours when incubated at 37 °C under static conditions in wastewater supplemented with 0.5% glucose, 0.1% KH2PO4, 0.05% KCl, and 0.05% MgSO4.7H2O. Similar decolorization studies reported *Pseudomonas aeruginosa* PAO1, *Stenotrophomonas maltophila*, and *Proteus mirabilis* to be highly efficient in melanoidin decolorization. *Pseudomonas sp.* was able to accomplish maximum decolorization of up to 56% and a 63% reduction

in the COD of the wasted wash at pH 6.8 -7.2, temperature 30-35 ºC, and glucose 0.4% (w/v) after 72 hours. The isolate's biodegradation of melanoidin pigments was validated by spectrophotometric and HPLC examination of the treated effluent. This method might be utilized to create an environmentally acceptable and reasonably priced biotechnology package for the bioremediation of wasted wash prior to disposal. *Pseudomonas fluorescens*, which was isolated from molasses-contaminated soil samples, decolorized up to 76% of molasses wastewater (MWW) samples in four days at 30°C in non-sterile settings [67].

Three *Bacillus* isolates *B. thuringiensis* (MTCC 4714), *B. brevis* (MTCC 4716), and *Bacillus sp.* (MTCC 6506) were observed to decolorize synthetic melanoidins, such as GGA, GAA, SGA, and SAA. In addition to the decolorization of all four melanoidins (10%, v/v), a significant decrease in the values of physicochemical parameters was observed. *Bacillus sp.* (MTCC 6506) and *B. brevis* (MTCC 4716) induced the most decolorization, followed by *B. thuringiensis* (MTCC 4714). There was 15% higher decolorization in the medium with glucose as the only carbon source than in the one with both carbon and nitrogen sources. In the presence of glucose as the only energy source, melanoidin SGA underwent the greatest amount of decolorization (50%) whereas melanoidin GAA underwent the least amount. Melanoidins can be broken down oxidatively by acetogenic bacteria. Acetogenic bacterial strain, strain No. BP103 exhibited decolorization yield of 76.4 ± 3.2% after 5 days of cultivation at 30 °C in molasses pigments medium that contained 3.0% glucose, 0.5% yeast extract, 0.1% KH2PO4, and 0.05% MgSO4.7H2O, with the pH set to 6.0 [68].

Three bacterial strains obtained from the activated sludge of a distillery wastewater treatment plant *Xanthomonas fragariae*, *Bacillus megaterium*, and *Bacillus cereus* in both free and immobilized form were used in batch studies to investigate the degradation of anaerobically digested distillery wastewater. Up to 48 hours, the removal of COD and color with all three strains increased with time; beyond that, only a little rise in COD and color removal efficiency was seen for up to 72 hours. Removal efficiency was rather stable for the next 120 hours after this. The maximal COD and color removal efficiency for both free and immobilized cells of all three strains ranged from 66 to 81% and 65 to 75%, respectively.

The capacity of several microorganisms to decolorize molasses wastewater in both thermophilic and anaerobic settings was evaluated. The best strain was determined to be MD-32, which was recently obtained from a soil sample. According to taxonomical research, the strain most closely resembles *B. smithii* and is a member of the genus *Bacillus*. Under anaerobic circumstances, the strain decolorized 35.5% of the molasses pigment in 20 days at 55°C; however, when grown aerobically, no decolorization activity was seen [69].

Fifty isolates exhibited decolorization activity on solid medium (clear zone), according to the results. In the liquid medium containing molasses pigments, the strains No. BP103 and No.13A exhibited the best decolorization activity among them. When yeast extract was used as the nitrogen source, the decolorization activity of strains No. 13A and BP103 was 80.50% and 82.00% respectively. Under ideal circumstances and medium compositions, the strains No. 13 A and BP103 had decolorization activities of 90.54% and 96.75 % respectively. Strain No. 13A and No. BP103 were identified as *Acetobacter aceti* [70].

Three thermotolerant bacterial isolates - *Brucellatritici (B3), Bacillus nitratireducens (B2),* and *Bacillus paramycoides* (B1) were shown to be melanoidin-decolorizing agents. These isolates were further optimized for decolorization under a range of nutritional and physicochemical conditions. After 40 hours of incubation under static circumstances with 0.5% glucose (w/v), 0.5% peptone (w/v), 0.05% MgSO4, and 0.01% KH2PO4 at a pH of 6.0*, B. nitratireducens* (B2) showed the greatest degree of decolorization (86%) of the three species at 40°C. A consortium of *Staphylococcus aureus* and *Serratia odoriferae* demonstrated the highest decolorization efficiency, achieving 89% [71].

A thermotolerant bacterial culture comprising *Pseudomonas aeruginosa, Bacillus subtilis,* and *B. cereus* was recovered from soil polluted with distillery effluent. During a short 24-hour incubation period, *B. subtilis* showed the highest degree of decolorization (85%) at 45°C utilizing relatively modest carbon (0.1%, w/v) and nitrogen sources (0.1%, w/v). *B. cereus* and *Pseudomonas sp*. both exhibited decolorization rates of 73% and 69% under optimal conditions. Withstanding temperatures ranging from 35 to 50°C without compromising the exponential growth phase, *B. subtilis* showed the highest thermotolerance. According to results from many analyses, the species *Bacillus* had the highest bioremediation efficacy when compared to other bacterial cultures.

Thirteen bacterial isolates from a bioreactor treating a mixture of municipal and molasses wastewater were analysed for their ability to degrade and decolorize melanoidins. The isolates were initially screened for manganese peroxidase activity and their growth potential in four synthetic melanoidin solutions with concentrations ranging from 3 to 7 gL^{-1}. Among them, three isolates showed potential for manganese peroxidase production: two strains of *Klebsiella* sp. (B2 and B3), one strain of

Escherichia coli (B4), and one strain of *Lactobacillus kefiri* (B1). These isolates exhibited high tolerance to synthetic melanoidins. 16S rDNA sequencing confirmed their close relation to *E. coli* and *Klebsiella sp.* [72].

A strain of *Streptococcus sp.* from a distillery near a natural environment and optimized it for decolorizing distillery effluent at different physico-chemical and nutritional levels [73]. These bacteria demonstrated the highest degree of decolorization, 87%, at 40˚C using modified GYPE Medium, which is 1% molasses medium (1%, Grade-C molasses, 0.2%, Yeast extract, 0.3%, Peptone, 0.05%, MgSO4, 0.05%, K2HPO4 with 3.5 OD effluent) pH-6.0 in 30 hours.

Among the 19 bacterial isolates that were recovered from a distillery sludge, strain *Bacillus albus* showed greater capacity to remove the color of the effluent. The bacteria caused up to 83% of the effluent to become significantly decolored [74]. Manganese peroxidase (MnP) produced by *Pseudoduganella violacea* demonstrated significant decolorization of Maillard products, achieving up to 83.68% at a temperature of 37°C within 192 hours of incubation holding potential for effective bioremediation applications to degrade Maillard products [75].

IV. NOVEL APPROACHES USING BACTERIAL STRAINS

Although several bacterial strains have been found and used to decolorize melanoidin, these strains' effectiveness and versatility are frequently restricted. Effective breakdown of melanoidin requires the employment of highly specialized or diversified enzymatic pathways due to its complicated structure, which comprises a variety of chemical linkages and a large molecular weight. Furthermore, variables such as pH, temperature, salinity, and the presence of other contaminants in industrial effluents might affect the activity of already recognized bacterial strains. This restriction emphasizes the necessity of investigating novel bacterial strains that are hardy and more effective in a range of industrial and environmental settings.

Discovering new bacterial strains may help find microorganisms with potential enzymatic properties, improved metabolic processes, and cooperative relationships with other living organisms. These strains could enable more efficient and cost-effective bioremediation processes, reducing dependency on chemical treatments that are often less sustainable and environmentally harmful. Furthermore, investigating novel strains may result in the development of consortia or modified microorganisms specifically suited for industrial effluents, increasing the viability and scalability of melanoidin decolorization procedures. In conclusion, investigating novel bacterial strains is essential to overcoming the drawbacks of current bioremediation techniques and improving the sustainability and effectiveness of melanoidin removal, which helps to preserve the environment and ensure that industries adhere to pollution regulations.

The use of microorganisms for melanoidin decolourisation offers numerous advantages in wastewater treatment. Microbial decolourisation is eco-friendly, as it avoids harmful chemicals, thereby reducing environmental impact. It is also cost-effective, utilizing naturally occurring microorganisms instead of expensive chemical reagents [76]. Many microorganisms, such as bacteria and fungi, have the capability to biodegrade melanoidin into less toxic compounds, enhancing the safety of treated water. Furthermore, microorganisms exhibit adaptability to diverse environmental conditions, making them effective in various wastewater scenarios. This process is sustainable, as microorganisms are renewable resources that can be maintained with minimal inputs. Certain strains also demonstrate specificity in targeting melanoidin, ensuring efficient decolourisation without affecting other components in the wastewater. Additionally, microbial degradation pathways can produce valuable by-products, contributing to resource recovery. The scalability of microbial treatments, from laboratory to industrial applications, and their minimal energy requirements make them versatile and cost-saving. Finally, microorganisms can be integrated with other treatment methods for improved decolourisation efficiency.

Integrating bacterial decolorization into distillery effluent treatment systems aligns with the principles of green chemistry and promotes sustainable development. This approach provides an effective solution to the twin challenges of pollution mitigation and resource optimization, making it a critical area of scientific inquiry.

V. TREATMENT USING NANOPARTICLES

Melanoidin decolourisation by utilizing biosynthesized silver nanoparticles and bacterial extract (*Bacillus sp*.) in an immobilised state, where the bacterial extracellular supernatant showed over 65% melanoidin decolorization (in 12 hours) [77]. On the other hand, biosynthesized AgNPs demonstrated 82% clearance under comparable circumstances. The greatest melanoidin elimination of 92% in 12 hours is achieved by the cell free extract immobilized with manufactured AgNPs; this highlights nano-coupled biomaterial immobilization as an appropriate method for quick melanoidin decolorization. ZnO nanoparticles possess significant potential for melanoidin adsorption,

offering an alternative method for treating colored effluents [78].

VI. CONCLUSION

Environmental impacts on the ecosystem due to discharge of untreated distillery wastewater could be addressed through improvised microbial bioremediation methods. Release of microbial decolorized distillery effluent could reduce the damaging effects of the wastewaters and could overcome the detrimental effects of physical and other chemical methods of treatments. The novel techniques that include use of microbial enzyme systems, immobilized bacterial consortia and use of nanoparticles in treating the distillery wastewater could be a cost-effective method. Combination methods using microbial consortium containing mixed cultures of potential melanoidin decolorizing microbes and nanoparticles holds great promise in treating the distillery effluents.

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