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MELANOIDIN DECOLORIZATION POTENTIAL OF INDIGENOUS BACTERIAL ISOLATES FROM DISTILLERY EFFLUENT

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ABSTRACT

Distillery industry generates large amounts of spent wash, containing melanoidin. Its resistance to biodegradation, high oxygen demand and toxicity is of critical concern due to its ecological impact on aquatic ecosystems, soil fertility, and crop viability. While conventional treatment methods are inefficient, microbial degradation offers a green, cost-effective alternative. This study aimed to isolate and screen melanoidin-decolorizing bacteria from distillery wastewater. Decolorization studies were carried out using synthetic melanoidin in four different media. Role of laccase and manganese peroxidase enzyme activities of the were evaluated. 16S rRNA sequencing revealed the isolates as *Bacillus subtilis*, *Enterobacter hormaechei* and *Priestia aryabhattai*. *P. aryabhattai* showed the highest melanoidin decolourization efficiency of 87.89% in minimal salt media, followed by *E. hormaechei* with 85.39% in nutrient broth, and *Bacillus subtilis* with 79.82% in minimal salt medium at 96 hours of incubation. All three strains demonstrated effective decolourization under optimal conditions of 37°C and pH 7. Tests on the germination of *Vigna radiata* seeds and on the growth of *Rhizobium leguminosarum* suggested the degraded products are non toxic. The findings demonstrate that the isolated strains have strong potential for effective and eco-friendly remediation of melanoidin in distillery wastewater, offering a valuable biotechnological tool for wastewater treatment.

Keywords: Melanoidin, distillery effluent, *Bacillus subtilis*, *Enterobacter hormaechei*, *Priestia aryabhattai*, wastewater treatment

INTRODUCTION

Distilleries are major industries in India that generate large volumes of wastewater, known as spent wash, which adversely affects soil and water quality. This brown effluent, generated during ethanol production, is rich in organic matter, nitrogen compounds, salts, and exhibits low pH, and elevated temperatures (Mikucka and Zielińska, 2020). For every litre of alcohol, 8-15 L of effluent is produced, rich in COD and BOD due to proteins, polysaccharides, polyphenols, waxes, and melanoidin (Saha *et al*., 2005; Melamane *et al*., 2007).

A major challenge in wastewater remediation is the colour caused by melanoidin, a dark brown polymer produced via the Maillard reaction between amino and carbonyl groups (Reynolds, 1968; Miura & Gomyo, 1982; Wedzicha & Kaputo, 1992; Kumar *et al*., 1997). Melanoidin absorbs sunlight, reducing photosynthesis in aquatic plants, lowering oxygen production, and leading to ecological imbalances like eutrophication and biodiversity loss (Jiranuntipon *et al*., 2008).

Conventional treatment methods, such as ozonization, flocculation, and activated carbon adsorption, are effective but costly and generate sludge (Patel *et al*., 2023). Nanomaterials like FeO and silver nanoparticles show promising results (Palani *et al*., 2015; Akhtar *et al*., 2024), but microbial degradation stands out as an eco-friendly, cost-effective alternative. Microorganisms such as bacteria, fungi, and algae degrade melanoidin through enzyme action, adsorption, and nutrient assimilation, utilizing enzymes like laccases, manganese peroxidase (MnP), lignin peroxidase (LiP), and sugar oxidases (Kumar *et al*., 1998; Cuoto *et al*., 2005; Freitas *et al*., 2009). Microbial decolorization offers numerous advantages such as, it is eco-friendly, cost-effective, sustainable, adaptable to various environmental conditions, and can produce valuable by-products. It aligns with green chemistry principles and supports sustainable development by providing an efficient, scalable, and environmentally responsible solution to melanoidin pollution in distillery wastewater (Sarma *et al*., 2025).

Considering the environmental concerns posed by melanoidin-rich distillery wastewater, the present study aimed to isolate and characterize melanoidin-decolorizing bacteria from distillery effluents and optimize parameters like pH, temperature, media, and incubation for enhanced efficiency. The enzymatic activities involved in degradation, ultimately contributing to a sustainable bioremediation strategy for industrial wastewater were also investigated.

MATERIALS AND METHODS

Collection of samples

Three samples were collected namely, distillery spent wash from a microbrewery (Bengaluru), textile dye-contaminated soils from Sualkuchi, Assam and Bengaluru, Karnataka. Samples were stored at 4°C, protected from light for further analysis.

Enrichment of the melanoidin decolorizing microorganisms

Ten mL of spent wash and ten grams of soil samples were added to nutrient broth containing 5% melanoidin and incubated at 37°C on a rotary shaker at 100 rpm for 24-48 hours.

Primary Screening of the Enriched Culture (Singh & Singh, 2020)

The enriched microbial cultures were plated on GPYE media containing 0.05% $MgSO_4.7H_20$, 0.01% KH_2PO_4, 0.1% yeast extract and 0.5% glucose and incubated at 37°C for 24 hours.

Preparation of synthetic melanoidin pigment and Optimization of λ_{max} (Dahiya *et al*., 2001)

Melanoidin was chemically synthesized in the lab. A solution of 2M D-Glucose, 2M Glycine and 0.2M Sodium bicarbonate was prepared with distilled water and was adjusted to pH 7.0 with 0.1N HCl. The solution was refluxed at 100°C for 2 hours, resulting in a brown-black liquid. The brown liquid was filtered using dialysis membrane against double distilled water for 24 hours. The resulting filtrate of synthetic melanoidin was lyophilized. λ_{max} was analyzed by UV-Visible Spectroscopy (200-800nm).

Secondary Screening of the Enriched Culture

- **Media Preparation:** Four different media were used for melanoidin decolorization studies: Nutrient Broth, GPYE (0.05% $MgSO_4.7H_20$, 0.01% KH_2PO_4, 0.1% yeast extract and 0.5% glucose), Minimal media (4g/L K_2HPO_4, 4g/L KH_2PO_4, 2g/L $(NH_4)_2SO_4$, 0.5g/L $MgSO_4.7H_2O$, 0.01g/L $CaCl_2$, 0.01g/L $FeSO_4.7H_2O$ and 0.1% Yeast Extract) and GPYM (1% Glucose, 0.05% Peptone, 0.1% K_2HPO_4, 0.05% $MgSO_4.7H_2O$).
- **Preparation of inoculum:** A loopful of enriched cultures were inoculated into 50 mL of four different media. The cultures were then incubated at 30°C and 37°C overnight on a rotary shaker at 100 rpm.
- **Decolourization Assay (Tiwari & Gaur, 2014)**: Overnight cultures (10%) were inoculated at into the media containing 5% synthetic melanoidin and were incubated at 30°C and 37°C up to 96 hours. The samples were centrifuged at 10,000 rpm for 10 minutes. The supernatant was collected. Absorbance at 475nm was recorded by UV-Vis Spectrophotometer.
- Decolourisation efficiency of isolates was calculated by the following formula [10]

$$\text{Decolourisation } \% = \frac{(\text{Initial aborbance} - \text{Final ab}}{\text{Final absorbance}}$$

- **FTIR Analysis:** FTIR analysis was conducted to compare the structural changes in melanoidin before and after treatment with isolates.

Molecular identification of the isolates and their phylogenetic analysis

Molecular identification by 16S rRNA sequencing was outsourced to Barcode BioSciences Pvt Ltd. The nucleotide sequence obtained from the 16S rRNA sequencing was analysed through NCBI BLAST by aligning homologous sequences. Based on the maximum identity score, first nine sequences were selected and aligned using multiple alignment software Clustal W. Distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA 12.

Optimization of physical parameters for melanoidin decolourisation

Media were inoculated with a 5% (v/v) microbial inoculum containing 5% synthetic melanoidin. To optimize temperature, melanoidin decolorization was studied at four different temperatures: 25°C, 30°C, 37°C and 45°C and pH at 4, 7 and 9. Decolorization was monitored at 24, 48, 72, and 96 hours of incubation.

Enzyme Assay

Manganese peroxidase activity:

Bacterial cultures were streaked onto GPYM media containing 5% melanoidin

and 0.1% phenol red (indicator) for manganese peroxidase (MnP) activity. The plates were incubated at 37°C for 24 hours to allow bacterial growth and enzyme expression. A yellow inhibition zone surrounding the bacterial colonies indicated the presence and activity of manganese peroxidase (Miyata *et al.*, 1998).

Laccase activity:

Laccase activity was assessed by monitoring the oxidation of guaiacol (o-methoxyphenol catechol monomethylether). The reaction mixture consisted of 1 mL of 1 mM guaiacol prepared in 0.1 M sodium phosphate buffer (pH 6.0) combined with 1 mL of crude enzyme extract. This mixture was incubated at 30°C for 10 minutes, and the oxidation of the substrate was tracked spectrophotometrically by measuring the increase in absorbance at 495 nm (Dhaliwal *et al*., 1991).

Antimicrobial Assay *Rhizobium sp.* was cultured on Muller-Hinton Agar using the swab method. Sterile discs loaded with treated supernatants from each isolate and control (untreated melanoidin) were placed on the agar surface and incubated for 24 hours. Zones of inhibition were measured (Roy *et al*., 2013).

Phytotoxicity Assay

Healthy seeds of *Vigna radiata* were surface sterilized with 0.1% $HgCl_2$ for 30 seconds and placed in Petri plates. The study evaluated the phytotoxicity of treated samples (supernatants of Isolate 6 RV, Isolate II, and Isolate III) compared to untreated melanoidin as a control (Mahla & Bhatt, 2022). Seed germination and root length (cm/seed) were monitored over a week.

Statistical Analysis: The decolourization experiment in this study was conducted in three replicates. The findings were reported as mean and standard deviation. Error bars were plotted.

RESULTS AND DISCUSSION

Isolation, screening and identification of the melanoidin decolorizing bacteria

Three potential isolates namely, Isolate 6 RV, II and III from distillery wastewater exhibiting good decolorization in primary screening by plate assay were selected for the study. They showed showed good growth on GPYE containing 5% melanoidin.

For the secondary screening, a quantitative approach was employed using four different media (NB, Minimal Salt, GPYM & GPYE) each containing 5% melanoidin. Decolorization percentage was assessed by observation of the color reduction spectrophotometrically at 475nm. The selected isolates were further evaluated for enhanced decolorization under various physicochemical and nutritional conditions. Results of the preliminary identification of the three bacterial strains done by morphological, biochemical characterization according to Bergey's manual are illustrated in Table 1.

Molecular identification of the isolates and their phylogenetic analysis

Based on morphological, biochemical, and molecular identification by 16S rRNA analysis followed by BLAST revealed Isolate 6 RV was *Bacillus subtilis* R8 (Accession No: *MH371779.1*), Isolate II as *Enterobacter hormaechei* BSWGM 5 (Accession No: *OQ410311.1*), and Isolate III as *Priestia aryabhattai* MVY-004 (Accession No: *KY882273.1*).

Optimization of temperature for melanoidin decolorization

The effect of temperature on melanoidin decolourisation by the three bacterial isolates was evaluated at 25°C, 30°C, 37°C, and 45°C. The results illustrated in figure 2 demonstrate that temperature plays a crucial role in influencing the decolourisation efficiency of the isolates in different media. At 25°C, all three isolates exhibited moderate decolourisation efficiency that was less than 50%. Isolate II showed the maximum decolourisation of 47.35% in GPYM at 96 hours, followed by Isolate 6 RV (43.09%) in GPYM and Isolate III (43%) in NB. The overall performance at this temperature suggests that while some decolourisation occurs, enzymatic activity may not be at its optimum, leading to relatively lower efficiencies.

At 30°C, a significant increase in decolourisation efficiency was observed, particularly for Isolate II, which exhibited the highest decolourisation of 80.89% in GPYM at 96 hours (Figure 3). Isolate 6 RV showed improved decolourisation in NB (42.41%) at 48 hours, while Isolate III displayed its highest decolourisation in NB (38.57%) at 48 hours. The increased efficiency at 30°C could be attributed to a temperature-induced enhancement in enzymatic activity and bacterial metabolism, promoting higher melanoidin degradation.

At 37°C, decolourisation percentage was the maximum for all isolates, as illustrated in Fig 4, Isolate III exhibited the highest decolourisation (87.89%) in MM at 96 hours, followed closely by Isolate II in NB (85.39%) and Isolate 6 RV in MM (79.82%). This suggests that 37°C is the optimal temperature for melanoidin degradation as evident in figure 5.

At 45°C, a drastic reduction in decolourisation efficiency was observed, except for Isolate II, which still exhibited relatively high decolourisation in MM (52.10%) at 48 hours (Fig 6b). Isolates 6 RV and III displayed considerably lower efficiencies, with Isolate 6 RV exhibit only 16.46% in NB and Isolate III achieving 18.83% in NB at 96 hours (Fig. 6). The sharp decline at 45°C suggests possible thermal denaturation of key enzymes involved in melanoidin breakdown, leading to reduced bacterial activity and decolourisation capacity.

The temperature dependent decline in decolorization activity can be attributed to either the loss of cellular viability or the denaturation of cellular enzymes (Pearce *et al*., 2003; Gomare *et al*., 2009). This phenomenon may be explained by the hypothesis proposed by Wong and Yuen (1996), which suggests that the accumulation of degradation products in the culture medium could negatively impact cell viability and decolorization efficiency. A reduction in decolorization activity at elevated temperatures, specifically at 45°C, may be associated with pH fluctuations during the process, which inhibit microbial growth and the degradation of colorants. These findings are consistent with a study reporting similar results on the decolorization of synthetic melanoidin containing wastewater (Jiranuntipon *et al*., 2008). Their study indicated that an increase in colour could result from the polymerization of melanoidins or the accumulation of toxic metabolites formed during the decolorization process,

leading to repression of decolorization activity.

Several studies have reported similar findings where various *Bacillus* strains exhibited an average decolorization efficiency of 75-81% under optimal conditions (Dahiya *et al*., 2001; Chavan *et al*., 2006; Kumar *et al*., 2008). Additionally, both studies identified pH 7 and 37°C as the optimal conditions for bacterial growth and decolorization, further reinforcing the idea that 37°C is the most suitable temperature for spent wash effluent treatment.

A similar melanoidin decolorization study reported a 51.4% decolorization by *Enterobacter sp*. (Mittal *et al*., 2018). In the current investigation, the same bacterial genera exhibited 85% melanoidin decolorization in nutrient broth. This substantial increase in decolorization efficiency highlights the strain's potential for melanoidin degradation. Overall, the findings indicate that the optimal temperature for melanoidin decolourisation by these isolates is 37°C, where the highest decolourisation efficiencies were observed.

Effect of pH on melanoidin decolourization

The decolourisation efficiency of melanoidin by bacterial isolates was evaluated at pH 4, 7, and 9 under optimized growth conditions. pH is a key environmental factor influencing bacterial metabolism, enzyme stability, and substrate availability, thereby affecting melanoidin decolourisation efficiency. The data reveal that neutral pH (pH 7) is generally more favourable for melanoidin degradation, particularly for Isolate III, which exhibited the highest decolourisation (87.99%) at 96 hours (Fig 7).

At pH 4, although all isolates demonstrated high decolourisation, a decline was observed at 96 hours, with Isolate 6 RV dropped from 77.25% at 72 hours to 68.01% and Isolate II from 78.67% to 73.34%. This suggests that while acidic conditions initially enhance decolourisation, prolonged exposure may lead to enzyme inhibition, reduced bacterial viability, or substrate depletion.

At pH 7, decolourisation was more sustained, with all isolates showing a steady increase over time. Isolate III exhibited the highest decolourisation at 96 hours (87.99%), followed by Isolate II (75.23%) and Isolate 6 RV (72.11%).The progressive increase without a decline suggests that neutral conditions provide an optimal balance between enzyme stability and bacterial metabolic activity. Many melanoidin-degrading enzymes, such as peroxidases and laccases, function optimally at neutral pH, which could explain the enhanced and prolonged decolourisation efficiency. Additionally, neutral pH supports bacterial cell integrity, optimal nutrient uptake, and enzyme secretion, all of which contribute to higher decolourisation rates.

At pH 9, decolourisation was significantly reduced in most isolates, particularly for Isolate II (7.81%) and Isolate III (14.93%) at 96 hours. The poor degradation at alkaline pH suggests enzyme instability, reduced bacterial growth, or lower substrate availability due to altered melanoidin solubility. This suggests that alkaline conditions negatively affect bacterial activity and enzyme function, potentially due to enzyme denaturation

or reduced bioavailability of melanoidin as a substrate.

Effect of different media on melanoidin decolourization

The effect of different growth media on melanoidin decolourisation by three bacterial isolates was investigated across varying temperatures. At 25°C, GPYM supported the highest decolourisation for Isolate 6 RV (43.09%) and Isolate II (47.35%), likely due to its rich nutrient profile enhancing enzymatic activity at lower temperatures. However, at elevated temperatures (30°C and 37°C), MM (Minimal Medium) and NB (Nutrient Broth) demonstrated superior decolourisation efficiencies, suggesting improved bacterial adaptability under thermophilic conditions. At 37°C, MM yielded the highest decolourisation for Isolate 6 RV (79.82%) and Isolate III (87.89%), while NB was optimal for Isolate II (85.39%). MM's composition, with melanoidin as the sole carbon and nitrogen source, likely induced specific melanoidin-degrading enzymes such as laccases and peroxidases, contributing to efficient polymer breakdown. In contrast, NB facilitated growth-associated decolourisation, especially in Isolate II, but lacked the targeted enzymatic induction observed in MM. These findings highlight MM's potential to enhance enzymatic activity and substrate specificity at higher temperatures, making it a promising medium for further optimization. Supporting this, a study by Rani and Pal (2013) reported maximum decolourisation of 51% and 52.5% by *Bacillus megaterium* (SW3) and *B. subtilis* (SW8), respectively, under optimized conditions (pH 7.2, 37°C, MM). In comparison, our study demonstrated higher decolourisation by *B. subtilis* (79%) and *P. aryabhattai* (87%) in MM at pH 7 within four days, indicating the superior efficiency of our isolates under similar conditions.

Effect of incubation period on melanoidin decolourization

The bacterial isolates demonstrated a progressive increase in melanoidin degradation with extended incubation periods, indicating active metabolic involvement in the decolorization process. However, beyond a certain incubation threshold, no significant enhancement in degradation efficiency was observed. This plateau in activity may be attributed to several factors, including the possible accumulation of secondary metabolites or by-products that exhibit inhibitory or toxic effects on microbial growth and enzymatic function. Additionally, the depletion of essential nutrients in the growth medium over prolonged incubation could have contributed to the decline in metabolic activity. Furthermore, the microbial isolates may have entered a stationary or decline phase, during which the production of degrading enzymes is reduced. These findings underscore the importance of optimizing incubation time to maximize degradation efficiency while preventing adverse effects caused by prolonged culture conditions.

Fourier Transform Infrared Spectroscopic analysis

The FTIR analysis of bacterial treated melanoidin revealed significant spectral variations in the region of 400-500nm when compared to the untreated melanoidin control (Fig. 8). These changes in peak intensity and shift in

absorption bands suggest potential alterations in the molecular composition of melanoidin following treatment with the microbial isolates. The FTIR results provide strong evidence for the involvement of microbial Isolates 6 RV, II, and III in the degradation of melanoidin, suggesting their potential application in bioremediation strategies for distillery effluents.

Manganese peroxidase activity

All the three Isolates demonstrated positive manganese peroxidase activity, as illustrated by the clearing zones after 24 and 48 hours. A significant increase in the diameter of the clear zones was observed at 48 hours as observed in figure 9.

Laccase activity: All three isolates demonstrated positive lactase activity, as indicated by an increase in absorbance relative to control. In the control setup, distilled water was added to guaiacol as the substrate, whereas the test samples contained the supernatant with guaiacol. The observed increase in absorbance in the test samples suggested enzymatic activity, as elevated product formation corresponds to higher enzymatic activity, serving as a qualitative assessment.

Microbial Activity Assay:

An antimicrobial assay of the treated melanoidin was conducted on *Rhizobium sp.* The control plates, where untreated melanoidin was added, exhibited a zone of inhibition. In the experimental setup, three replicates were performed for each treatment condition. In T1 plates, where melanoidin treated with Isolate 6 RV was added, no zone of inhibition was observed. Similarly, in T2 plates, where melanoidin treated with Isolate II was added, no inhibition zone was detected. Likewise, in the third set of replicates, where melanoidin treated with Isolate III was added, no zone of inhibition was observed.

TABLES:

Table 1: Biochemical characterization of the isolates

Biochemical Tests	Isolate 6 RV	Isolate II	Isolate III
Indole	-	-	-
Methyl Red	-	-	-
Voges Proskauer	-	-	-
Citrate Utilization	+	+	+
Oxidase	+	+	-
Catalase	+	+	+
Manganese Peroxidase	+	+	+
Starch Hydrolysis	+	-	+
Gelatin Hydrolysis	+	-	-

Table 2: Phytotoxicity Assay - *Vigna radiata*

Length (cm)	Control (Untreated)	T1	T2	T3
Radicle	1.9	7.6±0.02	8.62±0.05	8.53±0.05
Plumule	1.5	7.1±0.05	8.12±0.02	8.34±0.02

Mean±Std dev;n=6; T1- Melanoidin treated with Isolate 6 RV; T2- Melanoidin treated with Isolate II; T3- Melanoidin treated with Isolate III

FIGURES:

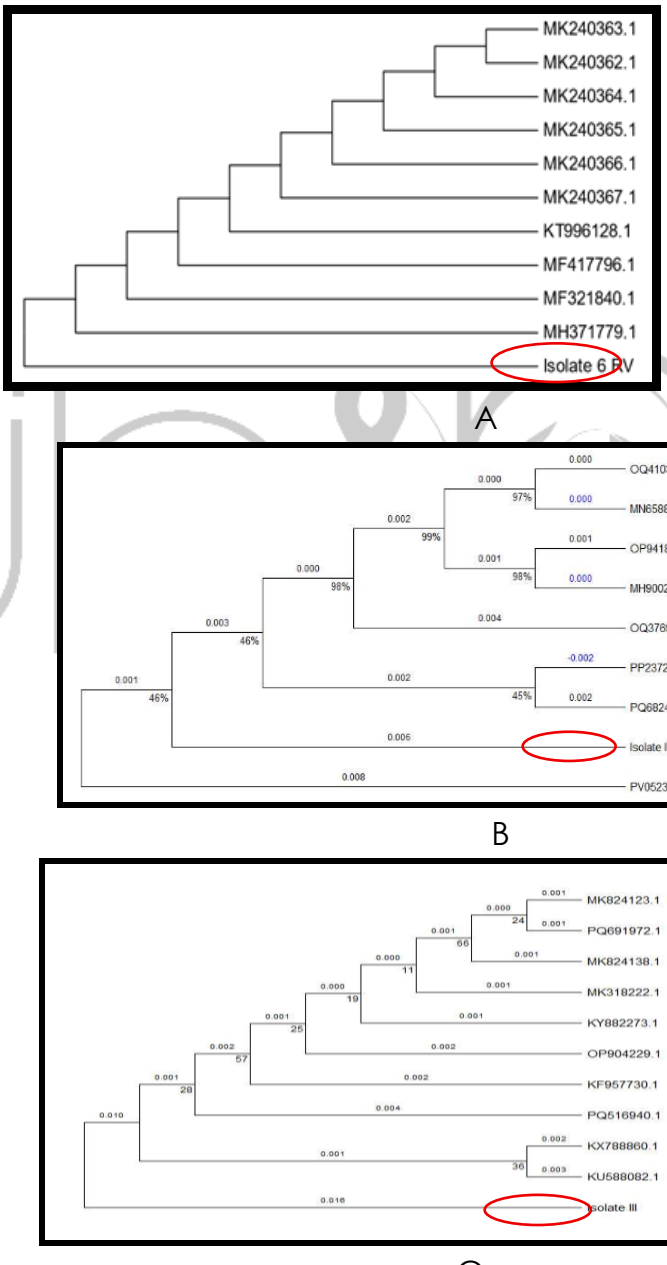

Fig. 1: Phylogenetic Tree **(A)** Isolate 6 RV (**B)** Isolate II (**C)** Isolate III

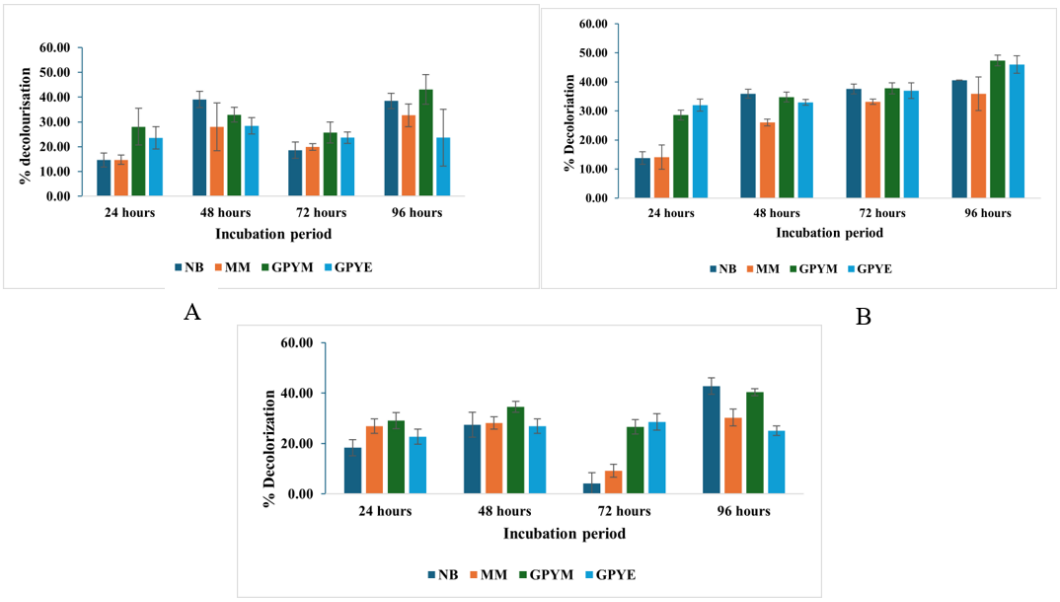

Fig. 2: Percent decolorization of melanoidin by the isolates at 25°C in different media at different incubation period **A.** Isolate 6 RV (*Bacillus subtilis*) **B.** Isolate II (*Enterobacter hormaechei*) **C.** Isolate III *(Priestia aryabhattai*)

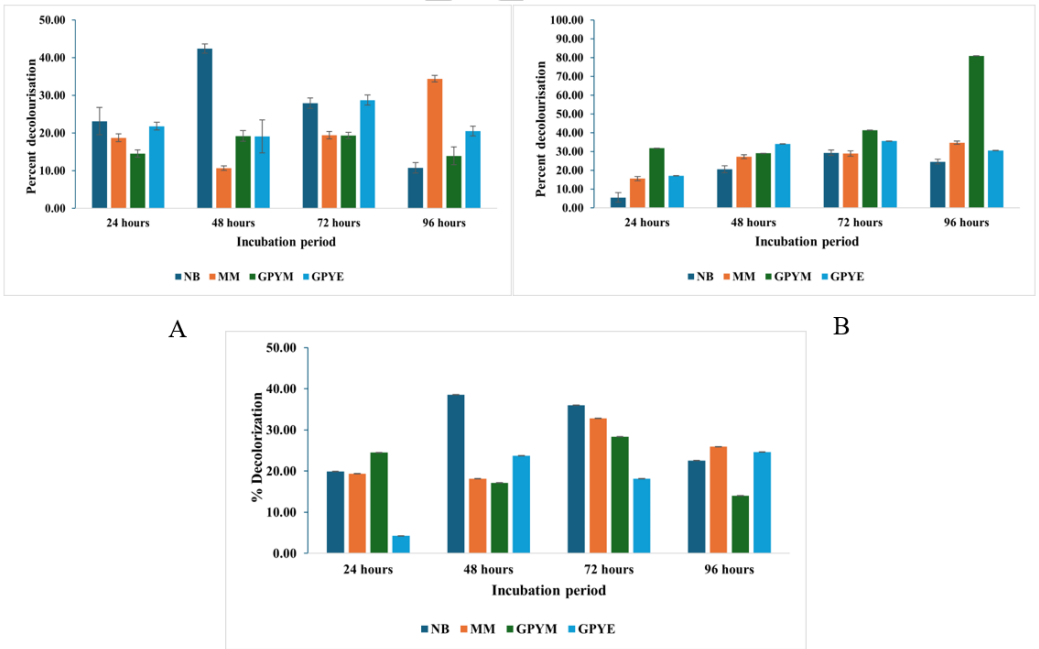

Fig. 3: Percent decolorization of melanoidin by the isolates at 30°C in different media at different incubation period **A.** Isolate 6 RV (*Bacillus subtilis*) **B.** Isolate II (*Enterobacter hormaechei*) **C.** Isolate III *(Priestia aryabhattai*)

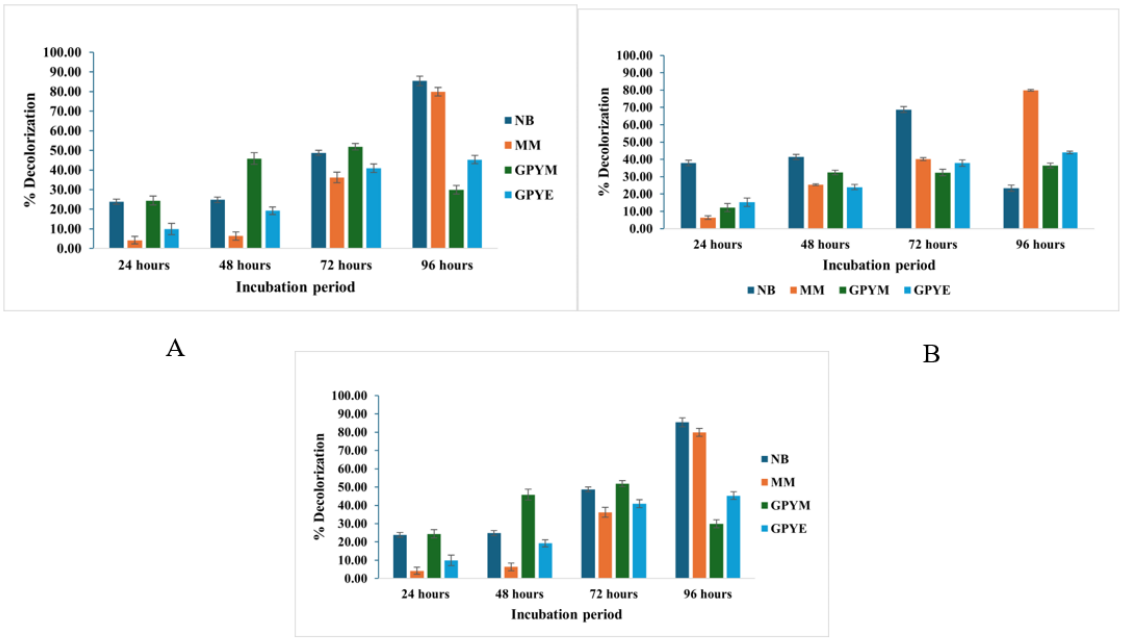

Fig. 4: Percent decolorization of melanoidin by the isolates at 37°C at different incubation period: **A.** Isolate 6 RV (*Bacillus subtilis*) **B.** Isolate II (*Enterobacter hormaechei*) **C.** Isolate III *(Priestia aryabhattai*)

Fig. 5: A. Untreated melanoidin; Melanoidin decolorization by *Priestia aryabhattai* mvy-004 at 37°C in MSM at different incubation time **B.** 24 hrs **C.** 48 hrs **D.** 72 hrs and **E.** 96 hrs

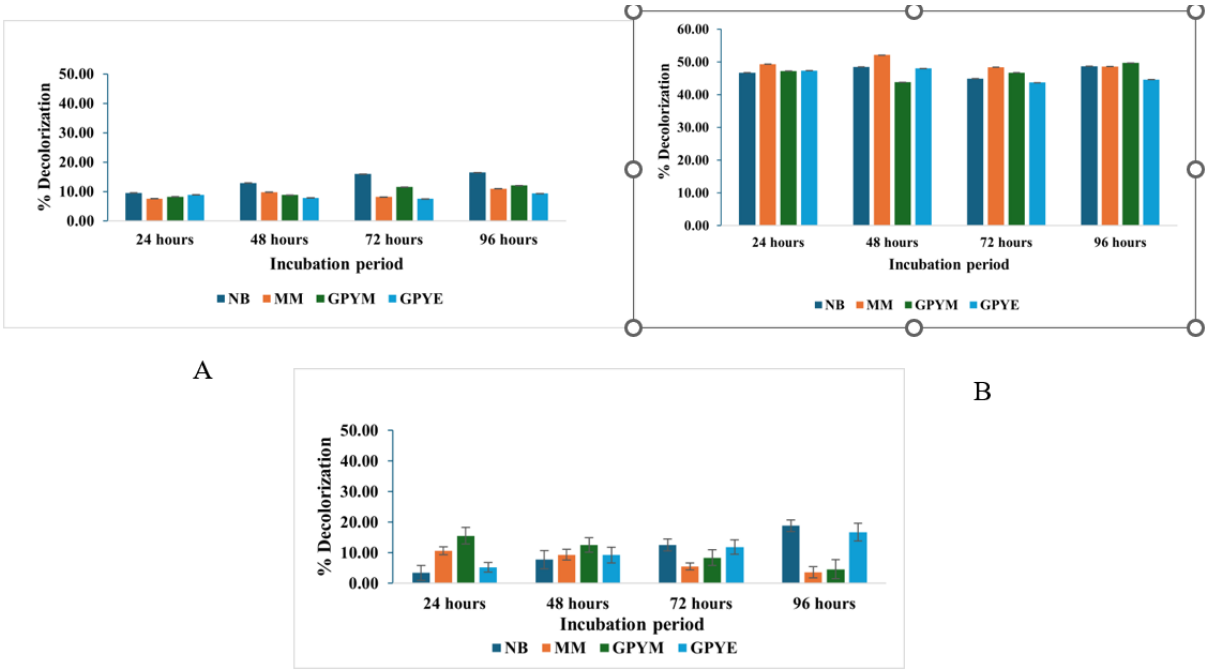

Fig. 6: Percent decolorization of melanoidin by the isolates at 45°C at different incubation period A. Isolate 6 RV (*Bacillus subtilis*) **B.** Isolate II (*Enterobacter hormaechei*) **C.** Isolate III *(Priestia aryabhattai*)

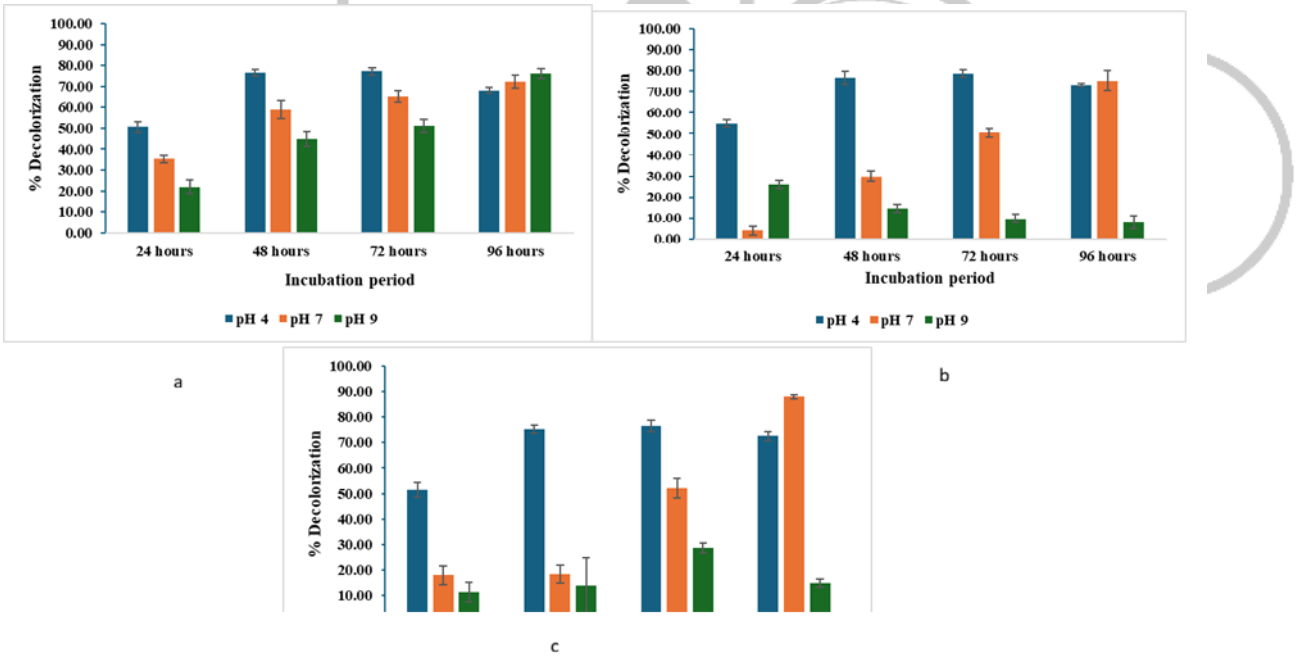

Fig. 7: Percent decolorization of melanoidin by the isolates at different pH
A. Isolate 6 RV (*Bacillus subtilis*) **B.** Isolate II (*Enterobacter hormaechei*) **C.** Isolate III *(Priestia aryabhattai*)

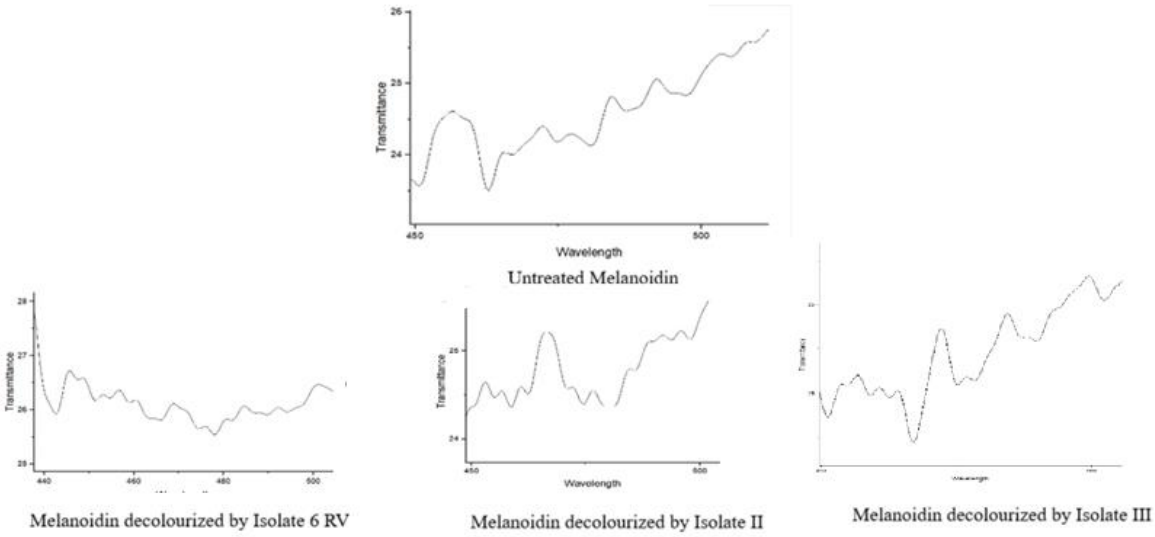

Fig. 8: Fourier Transform Infrared Spectroscopic analysis at 450-500nm

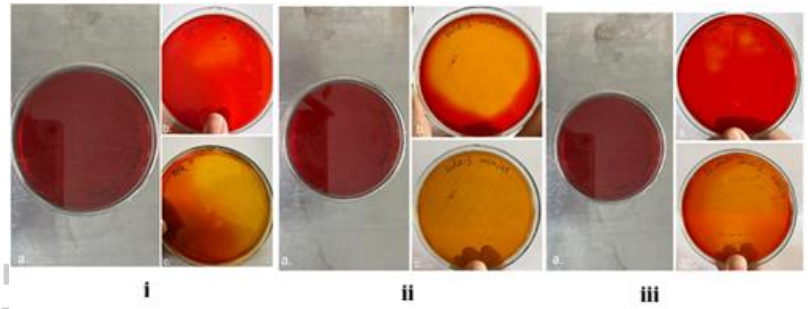

Fig. 9: Manganese peroxidase activity assay of (**A)** Control (i) *Bacillus subtilis* (ii) *Enterobacter hormaechei* (iii) *Priestia aryabhattai* at (**B)** 24 hours (**C)** 48 hours

Phytotoxicity Assay:

The phytotoxicity assay using *V. radiata* seeds demonstrated a significant improvement in seedling growth when treated with melanoidin degraded by bacterial isolates compared to the untreated control. In the control group (untreated melanoidin), radicle and plumule lengths were notably stunted (1.9 cm and 1.5 cm, respectively), indicating phytotoxic effects of raw melanoidin. In contrast, seeds treated with the biodegraded effluent exhibited enhanced growth. Among the treatments, Isolate II (T2) showed highest average radicle (8.62 cm) and plumule (8.12 cm) lengths, followed closely by Isolate III (T3) and Isolate 6 RV (T1). These results suggest that microbial treatment effectively reduces the toxicity of melanoidin and restores the suitability of the effluent for environmental discharge or reuse in agriculture.

SUMMARY AND CONCLUSION

This study explores the microbial decolorization of melanoidin from distillery spent wash using efficient bacterial strains. Primary and secondary screenings confirmed decolorization potential, with optimization experiments showing that temperature, pH, incubation time, and media composition significantly affected efficiency. The highest decolorization was observed under mesophilic conditions (37°C, pH 7). *P. aryabhattai* exhibited the highest decolorization (87.89%) in minimal salt media, followed by *E. hormaechei* (85.39% in nutrient broth) and *B. subtilis* (79.82% in minimal salt media), all within

96 hours. Enzymatic assays validated the presence of manganese peroxidase and laccase activity, suggesting the role of oxidative enzymatic pathways in melanoidin degradation. Similar study conducted also confirmed that the degradation of melanoidin was attributed to the enzymatic activity of manganese peroxidase and laccase in yeast (Singh *et al*., 2023).

Phytotoxicity and antimicrobial assays confirmed the detoxification of treated spent wash, with improved seed germination in *V. radiata*, highlighting the ecological benefits of microbial treatment. Hence this study effectively demonstrates the safe use of the of three potential bacterial distillery effluent isolates in decolorizing melanoidin from distillery spent wash, using synthetic melanoidin to assess their efficiency under controlled laboratory and industrial conditions.

Future research optimizing bioreactor conditions to enable continuous and efficient wastewater treatment processes with a focus on scalability could provide an efficient method to decolorize melanoidin using the three isolates investigated in this study. Additionally, genetic modification of *B. subtilis*, *E. hormaechei*, and *P. aryabhattai* holds significant potential to enhance their enzymatic activity, thereby improving melanoidin degradation across a range of environmental conditions. Incorporation of nanotechnology, such as enzyme-immobilized nanoparticles, could further augment decolorization efficiency by enhancing enzyme stability and catalytic performance. Developing eco-friendly microbial treatment strategies with minimal toxicity to aquatic ecosystems will be crucial to ensuring sustainable industrial wastewater management. A multidisciplinary approach integrating microbial engineering, nanotechnology, and process optimization could significantly enhance the efficiency and feasibility of bioremediation in industrial applications.

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