PROMINENT BACTERIAL ISOLATES FOR DECOLOURISATION OF DISTILLERY SPENT WASH

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KEYWORDS

Distillery spent wash, Melanoidins, Decolorization, Microbial isolate

ABSTRACT

Molasses-based distilleries produce large volumes of spent wash, a major environmental pollutant due to its high organic load and dark brown color. This coloration is primarily caused by melanoidins, which form through the Maillard reaction, a non-enzymatic process between sugars and amino acids. In this study, eight promising bacterial strains were selected from 40 isolates and designated as S1, S2, S3, S4, S5, S6, S7, and S8. These isolates were screened for their ability to decolorize distillery spent wash using both qualitative and quantitative analyses. Among them, isolate S5 exhibited the highest decolorization potential across different spent wash concentrations (10%, 20%, and 40%). Notably, at a 10% concentration, isolate S5 achieved complete (100%) decolorization, making it the most efficient strain in this study. Based on preliminary characterization, isolate S5 was tentatively identified as *Planococcus* species. Its exceptional decolorization ability suggests that it holds significant potential for commercial application in the bioremediation of distillery wastewater. Further research on optimizing environmental conditions and scaling up the process could pave the way for an eco-friendly and cost-effective solution to mitigate the environmental impact of distillery effluents.

INTRODUCTION

Molasses-based distilleries are major contributors to industrial pollution, generating vast amounts of high-strength wastewater with significantly elevated biochemical oxygen demand (BOD) and chemical oxygen demand (COD). One of the primary pollutants in distillery spent wash is melanoidin, a complex compound formed through the Maillard reaction—a non-enzymatic interaction between sugars and amino acids. Melanoidins are particularly concerning due to their structural stability, dark brown coloration, and strong odor, which contribute to environmental degradation by reducing light penetration in water bodies, altering microbial ecosystems, and inhibiting plant growth.[1] In this study, eight promising bacterial strains were selected from a total of 40 isolates and designated as S1, S2, S3, S4, S5, S6, S7, and S8. These isolates were screened for their ability to decolorize spent wash through both qualitative and quantitative analyses. Among them, isolate S5 demonstrated the highest decolorization potential at varying spent wash concentrations (10%, 20%, and 40%). Notably, at a 10% concentration, isolate S5 achieved 100% decolorization within a specified period, making it the most effective strain.

Preliminary identification classified isolate S5 as *Planococcus* species, highlighting its potential for commercial application in bioremediation. Given its efficiency, further research should focus on optimizing environmental parameters and scaling up the decolorization process for industrial applications. The successful implementation of such microbial approaches could provide a

sustainable, eco-friendly solution for mitigating the severe environmental impact of distillery wastewater.[2]

Various treatment methods, including anaerobic, aerobic, and physicochemical processes, have been explored to mitigate the environmental impact of distillery wastewater. Among these, anaerobic treatment is considered highly effective as a primary approach, achieving over 80% BOD reduction while also facilitating energy recovery [3]. However, complete decolorization remains a challenge, as untreated melanoidins contribute to water pollution and aesthetic degradation.

This study aims to isolate and identify microorganisms from soil capable of decolorizing distillery spent wash. The presence of color in wastewater before disposal into natural water bodies is undesirable, necessitating efficient biological treatment methods. By identifying and utilizing microorganisms with the ability to break down melanoidins, this research seeks to develop a sustainable and eco-friendly approach to mitigating pollution from distillery effluents.[4]

MATERIAL AND METHODS

Collection of distilleries spent wash sample:

Three spent wash samples were obtained from the distillery unit of Sahyadri Sahakari Sakhar Karkhana Ltd., located in YashwantNagar, Tal-Karad, Dist. Satara, Maharashtra. These samples were collected from the primary treatment facility of the distillery industry using sterilized plastic bottles to ensure contamination-free handling.

The collected samples were analyzed for various physico-chemical parameters, including color, odor, temperature, pH, Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), total solids, total dissolved solids (TDS), and total suspended solids. The analyses were conducted following the **Standard Methods for the Examination of Water and Wastewater** (APHA, 1995) to ensure accuracy and reliability of the results.

Collection of soil samples:

Total four soil samples were collected. Soil sample one was collected from distillery unit of Sahyadri Sahakari Sakhar Karkhana Ltd., YashwantNagar, Tal-Karad, Dist-Satara, State-Maharashtra. Soil sample second was collected from saw mill, near the Dhebewadi Fata, Karad, and soil sample third was spent wash spreaded soil from farm. Soil sample fourth was collected from Molasses spreaded soil from farm**.**

Enrichment and Isolation microorganisms from soil:

Microorganisms capable of decolorizing distillery spent wash were isolated using the enrichment culture technique. The isolation process was carried out using a minimal salt glucose medium containing 0.5% glucose, 0.01% ammonium phosphate, 0.05% sodium chloride, 0.02% magnesium chloride, and 0.01% dibasic potassium phosphate.[5] To enhance the growth of spent wash-decolorizing bacteria, the medium was supplemented with 10% spent wash, providing a selective environment for microbial adaptation and enrichment.

The spread plate technique was employed for bacterial isolation using agar medium. This method allowed for the selection of bacterial colonies that exhibited efficient decolorization zones, indicating their potential for melanoidin degradation. Bacterial colonies that demonstrated significant decolorization activity were further screened for their ability to degrade and remove color from distillery spent wash through qualitative and quantitative assessments.[6]

The ability of microorganisms to decolorize spent wash is crucial for the development of eco-friendly bioremediation strategies. Identifying and optimizing bacterial strains with high decolorization efficiency could provide a sustainable alternative to conventional wastewater treatment methods.[7] Further research will focus on characterizing these isolates, optimizing environmental conditions such as pH, temperature, and incubation period, and evaluating their

large-scale application for industrial wastewater treatment. The use of microbial approaches in treating distillery effluents holds promise for reducing environmental pollution and mitigating the toxic effects of melanoidins on aquatic and terrestrial ecosystems.[8]

Screening of the isolates for decolorization of distillery spentwash:

1) **Qualitative study for decolorization of distillery spent wash by isolates:**

   All bacterial isolates were spot inoculated onto Minimal Salt Glucose agar medium containing 10%, 20%, 40%, and 60% spent wash. The plates were incubated at 30°C for 24 to 48 hours to assess their decolorization potential. Bacterial colonies that demonstrated significant decolorization, evident by clear zones around them due to the breakdown of melanoidin pigment, were selected for further study.[9]

   The selected colonies were then subjected to repeated streaking on fresh agar plates to ensure purity and isolate distinct bacterial strains. This purification process helped in obtaining individual strains with high decolorization efficiency for further characterization. The identification and optimization of these strains could contribute to the development of effective bioremediation strategies for treating distillery wastewater. Future studies will focus on enhancing their decolorization capabilities and evaluating their potential for large-scale wastewater treatment applications.[10]

2) **Quantitative study for decolorization of distillery spent wash by isolates:**

   For the quantitative assessment of melanoidin-decolorizing bacterial isolates, they were inoculated into Minimal Salt Glucose broth containing varying concentrations of spent wash (10% and 20%). The flasks were then incubated at 30°C for a duration of 24 to 72 hours. At specific time intervals (0, 24, 48, and 72 hours), the broth samples were centrifuged at 10,000 rpm for 10 minutes to analyze decolorization efficiency.

The supernatant from the centrifuged samples was analyzed for absorbance at 475 nm using a spectrophotometer. The extent of decolorization was determined by measuring the reduction in absorbance at 475 nm compared to the initial absorbance at the same wavelength. An uninoculated medium was used as a control. The decolorization efficiency of the bacterial isolates was calculated using the following equation [8, 2]:

$$\%\text{Decolonization} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorption}} \times 100$$

RESULTS AND DISCUSSION

Physico-chemical analysis of distillery spent wash:

The physico-chemical analysis of the distillery spent wash revealed that it was highly acidic, with a pH of 5.9, a burnt sugar-like odor, and a distinct brown color. The concentrations of Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Dissolved Solids (TDS), and Total Suspended Solids (TSS) exceeded permissible limits, as shown in Table 1.

Table. 1 Physico-chemical properties of distillery spent wash

Sr.No	Physical Parameters	Average Values	Permissible limit (MPCB)
1	pH	5.9	5.5-8.8
2	Colour	Brown	-
3	Odour	Burnt sugar	-
4	Temperature	57.8°C	40-45
5	Total Solids	99400 mg/L	
6	Total Dissolved Solids	95490 mg/L	2100
7	Total Suspended Solids	3910 mg/L	100
8	Chemical Oxygen Demand (COD)	1,02,000 mg/L	250
9	Biological Oxygen Demand (BOD)	43,963mg/L	30-100

Isolation, Screening and identification of distillery spent wash decolorizing bacteria:

A total of four soil samples were collected from various natural sources for the isolation of distillery spent wash decolorizing bacteria. Eight bacterial isolates were obtained using a minimal salt glucose medium containing 10% spent wash and were designated as S1, S2, S3, S4, S5, S6, S7, and S8. These isolates were then screened for their ability to decolorize distillery spent wash through qualitative and quantitative methods (Figure-1).

During qualitative screening, all isolates were incubated in minimal salt medium (MSM) containing 10%, 20%, 40%, and 60% spent wash at room temperature for 24 to 48 hours (Figure-1). Among them, three isolates—S5, S6, and S8—demonstrated significant growth in 10% and 20% spent wash MSM medium, as indicated by the formation of a clear zone around the colonies (Table-2). These three isolates were further analyzed for quantitative decolorization efficiency.

In quantitative studies, S5, S6, and S8 were individually inoculated in MSM medium containing 10% and 20% spent wash and incubated for 24 to 72 hours. Among them, isolate S5 exhibited the highest melanoidin degradation, achieving 15.51% decolorization in 10% spent wash MSM broth, as confirmed by spectrophotometric analysis (Tables 2 & 4). Based on morphological, cultural, and biochemical characteristics, isolate S5 was tentatively identified as a promising candidate for further study (Table 3).

Figure 1. Percent decolorization at various concentrations of spent

Table 2 Qualitative study for decolorization of distillery spent wash

Bacterial isolates	Decolorization at different percentage of distillery spent wash concentration			
	10 %	20 %	40 %	60 %
S 1	+ +	+	+	-
S 2	+ +	+ +	+	-
S 3	+ +	+	+	-
S 4	+ +	+ +	+	-
S 5	+ + & DC	+ +	+	+
S 6	+ +&DC	+ +	+	-
S 7	++	+	+	-
S 8	+ +&DC	+ +	+	-

- : No growth
+ : Moderate growth
+ + : Significant growth
DC: Decolorization obtained

Table 3: Quantitative study for decolorization of distillery spent wash by promising isolates in liquid media (measurement of absorbance).

Isolates	Absorbance Density at 475nm wavelength							
	10%				20%			
	0h	24h	48h	72h	0h	24h	48h	72h
S5	0.58	0.15	0.10	0.00	1.04	0.58	0.44	0.31
S6	0.62	0.29	0.16	0.13	1.12	0.77	0.67	0.57
S8	0.59	0.35	0.22	0.15	1.07	0.88	0.98	0.64

Table 4. Percent Decolorization of distillery spent wash by promising isolates in liquid media

Isolates	% Decolorization	
	10%	**20%**
S5	100	70.19
S6	79.03	49.10
S8	74.57	40.18

CONCLUSION

In this study, various physicochemical analyses of distillery spent wash were conducted to enhance melanoidin decolorization using soil isolates. A total of eight bacterial isolates were obtained from soil samples, among which isolates S5, S6, and S8 demonstrated positive results in both qualitative and quantitative decolorization tests. Among these, isolate S5 exhibited the highest decolorization efficiency, achieving 100% decolorization of distillery spent wash within 72 hours. Based on preliminary identification, isolate S5 was tentatively classified as *Planococcus* spp. The promising results indicate that isolate S5 has significant potential for industrial wastewater treatment, particularly in the removal of melanoidin, a major pollutant in distillery effluents. Further research focusing on optimizing media composition and environmental conditions, such as pH, temperature, and incubation period, is necessary to enhance the decolorization efficiency of bacterial isolates. Understanding these factors will aid in developing an efficient and sustainable bioremediation strategy for distillery wastewater treatment. This study highlights the potential application of microbial approaches for mitigating environmental pollution caused by industrial effluents, offering a cost-effective and eco-friendly alternative to conventional treatment methods.

ACKNOWLEDGEMENT

The authors are grateful to the Honorable Chancellor, KVV, Deemed to be University, Karad for the valuable support and Dean, KIAS for providing all the research facilities to conduct the work.

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