Int.J.Curr.Microbiol.App.Sci (2023) *12*(10): 63-73

International Journal of Current Microbiology and Applied Sciences
ISSN: 2319-7706 **Volume 12 Number 10 (2023)**
Journal homepage: http://www.ijcmas.com

Original Research Article https://doi.org/10.20546/ijcmas.2023.1210.008

Decolourization of Spent Wash using Bacteria *Pseudomonas putida*

Ajad Patel[1], Ranjan Singh[1*], Abhishek Vashishtha[2], Soni Tiwari[1], Prabhash Kumar Pandey[4], Vijay Kumar Shukla[5], Laxmi Kant Pandey[6], Femina Sobin[7], Tanim Arpit Singh[8], Trashi Agrah Singh[9], Neeraj Khare[10], Shipra Singh[11], Rajeeva Gaur[1] and Mahendra Kumar Gupta[12]

[1]Department of Microbiology, Faculty of Science, Dr. Rammanohar Lohia Avadh University, Ayodhya, Uttar Pradesh, India
[2]Department of Microbiology, Maharaja Ganga Singh University, Bikaner, Rajasthan, India.
[3]Department of Soil Science and Agriculture Chemistry, Institute of Agriculture Science, Banaras Hindu University, Varanasi, Uttar Pradesh, India
[4]Center for Advance Research, Faculty of Medicine, King George's Medical University, Lucknow, Uttar Pradesh, India
[5]Department of Biochemistry, Faculty of Science, Dr. Rammanohar Lohia Avadh University, Ayodhya, Uttar Pradesh, India
[6]Department of Biotechnology, St. Aloysius College (Autonomous), Jabalpur, Madhya Pradesh, India
[7]Department of Botany and Microbiology, St. Aloysius College (Autonomous), Jabalpur, Madhya Pradesh, India.
[8]Maharaja Ranjit Singh College of Professional Sciences, Indore, Madhya Pradesh, India.
[9]Bhagyoday Tirth College of Paramedical Science, Sagar, Madhya Pradesh, India
[10]Institute of Allied Medical Science and Technology, NIET, NIMS University, Jaipur, Rajasthan, India
[11]Department of Microbiology, RRPG College, Amethi, U.P., India
[12]School of Studies in Botany, Jiwaji University, Gwalior, Madhya Pradesh, India

**Corresponding author*

Keywords

Decolourization, spent wash, bacteria, culture conditions, fermentation, sugar plants

Article Info

Received:
28 August 2023
Accepted:
25 September 2023
Available Online:
10 October 2023

A B S T R A C T

With extremely high BOD, COD, and other harmful organic and inorganic elements, spent wash is among the most complicated and time-consuming wastewaters. Because of the presence of several water soluble, colouring chemicals and recalcitrant primarily melanoidins, distillery effluent maintains a very dark brown hue even after anaerobic treatment. The aim of the study includes standardising several criteria for the extraction of melanoidin pigment as well as the isolation and identification of bacteria from natural sources. Methods: *Pseudomonas putida*, a melanoidin-decolorizing bacterium, was isolated in a lab setting using the serial dilution technique, and the culture conditions were improved at different incubation temperatures, times, pH levels, carbon sources, and nitrogen sources. On 120 hours, or the fifth day of culture, the optimal decolorization (86.05%) of melanoidins were accomplished at pH 5 and 37 °C. Based on the results of optimising the culture settings, it was discovered that the chosen bacterial strain needs 1.5 gm of extra carbon (Dextrose) and 1.5 gm of supplementary nitrogen (Ammonium sulphate) in order to decolorize. After bacterial treatment, dextrose and ammonium sulphate are supplied to the effluent, which is both cost- and environmentally-friendly. By using bacterial strain *Pseudomonas putida*, this method of biological treatment successfully decolorizes melanoidin from distillery effluent.

Introduction

The sugar factory produces molasses as a by-product. Alcohol is created when molasses has undergone fermentation. The effluent is created during the distillation of alcohol. It's referred to as wasted wash. It is a substance with colour and is harmful to the environment. Due to its colour and composition, wasted wash is a serious difficulty for disposal.

This issue has led to the closure of several industries. Some enterprises discharge their wastewater into rivers, which is problematic for those who live near rivers (Ravikumar, 2015). Some sugar plants create foul-smelling puddles of discarded wash.

Numerous research organisations have been working on this issue for many years, but no good answer has been found. Consequently, biological decolorization is required (Naik *et al.*, 2010; Singh *et al.*, 2007).

Waste water undergoes biological treatment to remove colour.

Effluent that has undergone microbial treatment could be less harmful and secure (Singh *et al.*, 2007). Due to the excessive chemical use caused by the application of physical and chemical processes, a significant quantity of sludge may be produced (Prescott and Dunn, 1979). These techniques have certain downsides, including the production of toxic by-products and high energy usage (Chavan *et al.*, 2006).

Literature Review

According to Santal *et al.*, (2016) and Gaikwad and Pisal (2016), spent wash decolourization research was conducted (Santal *et al.*, 2016; Gaikwad and Pisal, 2016). Shinde and Nakade (2021), looked at the function of microbes in the breakdown of used laundry (Khandekar and Shinkar, 2020).

Khandekar and Shinkar (2020) discovered certain microorganisms to be effective at removing the colour from meanoidins (Shinde and Nakade, 2021). Three distinct bacterial strains were first evaluated in liquid cultures by Duraisamy *et al.*, (2017), and then they were employed as a consortium for biosorption tests that revealed various decolorization abilities (Duraisamy *et al.*, 2017).

The main objectives of this research was carried out to find, identify, and test the efficacy of a microbe from a natural source in the decolorization of melanoidin identified in distillery effluent. Additionally, standardisation of several criteria for the chosen microorganism's removal of the melanoidin pigment.

Materials and Methods

Sample collection

The different samples were collected from India KM Sugar Mills, Ayodhya (Masuadha) in sterile polybags and bottles. Spent wash from the distillery was collected in sterile bottle. Soil from the nearby area of the distillery was collected in sterile bag.

Determination of physical parameters

Color

Color was noted visually.

Odour

Odour was determined by smelling.

pH

pH of the sample was measured by a pH meter.

Temperature

Temperature was taken in ºC at the time of sampling by simple manual thermometer.

Determination of chemical parameters

Determination of Total Alkalinity Phenolphthalein Method

The effluent sample of 50 ml was taken in conical flask and added two drops of phenolphthalein indicator, if pink color appears then titrated against 0.02 N (HCl) units the pink color disappeared.

Methyl orange Method

The same sample used for phenolphthalein alkalinity, was further used after disappearing of pink color in methyle orange test. Two drop of methyl orange indicator were added and titrated against 0.02N HCl until the color changed to orange.

Determination of Chloride

It was determined by argenometric method. Two ml of potassium chromate indicator solution was added to 100 ml of effluent sample in a beaker. It was then titrated against 0.014 N silver nitrate to pinkish end point.

Determination of nitrate-nitrogen

It was determined colorometrically by phenoldisulphonic acid method.

Isolation of bacterial strain

Serial Dilution Method

A serial dilution is a sequence of repeated dilutions used to lower the cell concentration of a dense culture. Each dilution would decrease the bacterium concentration by a particular amount.

Determining Optimum Temperature, pH and Effluent Concentration of Selected Bacterial Isolates

Temperature

Effect of temperature on bacterial growth was evaluated on NAM. Inoculated plates have been placed within the plastic bags and then incubated at 5, 28, 32 and 37°C in dark.

pH

To find out the optimal pH for the maximum decolourization by the chosen microorganism, the pH of Distillery Effluent was modified to different levels. The optimized Carbon and Nitrogen source were also added to Distillery Effluent. The pH was modified to 4.0, 6.0, 7.0 and 9.0 for bacterial with the help of pH buffer tablets.

Effect of Carbon Source for Maximum Decolourization

The Distillery Effluent decolourization by bacterial isolates was compared using different carbon source. Different carbon source like Dextrose, Lactose, Maltose, Sucrose, Starch was examined for the effects on decolourization.

Effect of Nitrogen Source for Maximum Decolourization

Different nitrogen sources like Ammonium sulphate, Yeast extract, Peptone, Sodium chloride, Beef extract have been tested for the impacts on the decolourization of Distillery Effluent by the chosen microorganism.

Effect of Different Concentration of Dextrose on Decolourization of Effluent

Availability of carbon source is also very important factor for microbial activities. Therefore, different levels of Dextrose (0.0%, 0.5%, 1.0%, 1.5%, 2.0%, 2.5%, and 3.0%) have been supplemented to Distillery Effluents and their effects on removal of the melanoidin pigment pollutant were recorded.

Effect of Different Concentration of Ammonium sulphate on Decolourization of Effluent

Different levels of Ammonium sulphate (0.0%, 0.5%, 1.0%, 1.5%, 2.0%, 2.5% and 3.0%) were

supplemented to Distillery Effluents and their effects on decolourization were assessed by adding Dextrose (1.5%) as the additional carbon source.

Results and Discussion

Physio-Chemical Characterization of Distillery Effluent

Fresh distillery effluent sample were collected from the samples were characterized for colour, odour and pH. The effluent was dark reddish-brown appearance with strong unpleasant colour having pH of 7.8.

Identification

Out of 4 bacterial strains isolated the best strain which showed the maximum decolourization was sent for identified to Centre for Medical Diagnostic and Research (CMDR), Motilal Nehru National Institute of Technology, Allahabad (U.P.).

The strain was identified as *Pseudomonas putida*. Therefore, further studies were carried out on this selected bacterial strain only (Physio-Chemical method).

Screening of Bacterial Isolates for Removal of Melanoidin from Distillery Effluent

Screening for potential strain is one of the most important and first step in the melanoidin removal. Out of the four bacterial strain only *Pseudomonas putida* showed highest rate of decolourization of melanoidin.

Effect of Temperature and Time on Decolourization of Melanoidin by Selected Bacterial Strain

Temperature plays very important role in bacterial growth. Optimum condition of temperature varies from species to species. Radial bacterial growth varied with temperature. In general, maximum radial growth of the bacterial strains was recorded at 37°C. Too much variation in range of temperature did not support the expansion of the isolated bacteria. Time plays a remarkable role in the growth of an organism. Maximum growth was observed at 120h. Further increase in the temperature reduced the growth of bacteria.

Impact of pH on Decolourization of Melanoidin by the chosen Bacterial Strain

Hydrogen ion concentration of the medium is another very important parameter which is related with the progress and activities of the organism. The effects of different pH on colour reduction were assessed. The colour reduction was highest at pH 5.0. Maximum colour reduction was achieved by *Pseudomonas putida*.

Impact of various Carbon Sources on the Decolourization of the Melanoidin by Selected Bacterial Strain

Carbon source plays a vital role in decolourization of distillery effluent. Table 3 depicts different carbon source namely- Dextrose, Lactose, Maltose, Sucrose and Starch were studied for melanoidin decolourization. The level of decolourization varied significantly with various carbon sources. In general Dextrose supported maximum colour reduction by the bacterial isolates (70.75%) at a concentration of 1.5 gm. Lactose was ought to be the next carbon source with 38% decolourization by the bacteria.

Impact of Various Concentrations of the Dextrose on Decolourization of the Melanoidin by Selected Bacterial Growth

Further different levels of Dextrose (0.5gm, 1.0gm, 1.5gm, 2.0gm, 2.5gm and 3.0gm) were supplemented to the distillery effluent as well as their impacts on removal of the melanoidin pigment were recorded. It was observed from the table that maximum decolourization was observed at 1.5 gm of dextrose. Further increase or decrease in the concentration of Starch did not support the decolourization of distillery effluent.

Table.1 Impact of different temperature and time on the selected strain of bacterial growth.

Days	5°C	28°C	32°C	37°C	40°C
Day/hrs.	(cm.)	(cm.)	(cm.)	(cm.)	(cm.)
Day 0 (0hrs.)	0.0	0.0	0.0	0.0	0.0
Day – 1(24hrs.)	0	0.2	1.3	1	0.5
Day – 2 (48hrs.)	0.3	1.6	1.8	2	1.0
Day – 3(72hrs.)	0.3	2.6	2.2	2.6	2
Day – 4(96hrs.)	0.3	2.5	2.3	2.8	1.8
Day – 5(120hrs.)	0.3	2.0	2.5	3.0	2.5
Day – 6 (144 hrs.)	0.3	1.5	2.0	2.0	1.5
Day – 7 (168 hrs.)	0.3	1.0	1.5	1.0	0.5

Table.2 Impact of different pH on decolourization of melanoidin by selected bacterial strain.

pH	Decolourization (%)
4.0	77.44
4.5	80.55
5.0	86.50
5.5	85.55
6.0	84.55
6.5	83.65
7	82.65

Table.3 Impact of various carbon sources on the decolourization of the melanoidin by selected bacterial strain.

S. No.	Source of Carbon	O.D. (nm)	Decolourization (%)
1	Dextrose	0.078	70.75
2	Maltose	0.205	24.67
3	Lactose	0.178	38
4	Starch	0.242	23.25
5	Sucrose	0.174	36.29

Table.4 Impact of different concentration of dextrose on decolourization of melanoidin by selected bacterial strain

S. No.	Concentration	O.D. (nm)	Decolourization (%)
1	0.5	0.052	60.75
2	1.0	0.061	66.50
3	1.5	0.078	70.75
4	2.0	0.075	66.50
5	2.5	0.069	60.55
6	3.0	0.065	57.55

Table.5 Impact of various nitrogen source on decolourization of melanoidin by selected bacterial strain.

S. No.	Nitrogen Source (1.5g)	O.D. (nm)	Decolourization (%)
1	Beef extract	0.150	44.80
2	Yeast extract	0.174	39
3	Peptone	0.169	40.07
4	Sodium Chloride	0.155	43.74
5	Ammonium Sulphate	0.145	48.58

Table.6 Impact of different concentration of ammonium sulphate on decolourization of melanoidin by selected bacterial strain.

S. No.	Concentration	O.D. (nm)	Decolourization (%)
1	0.5	0.025	43.50
2	1.0	0.050	44.55
3	1.5	0.145	48.58
4	2.0	0.130	46.75
5	2.5	0.080	42.50
6	3.0	0.070	39.75

Fig.1 Impact of different temperature and time period on selected bacterial growth.

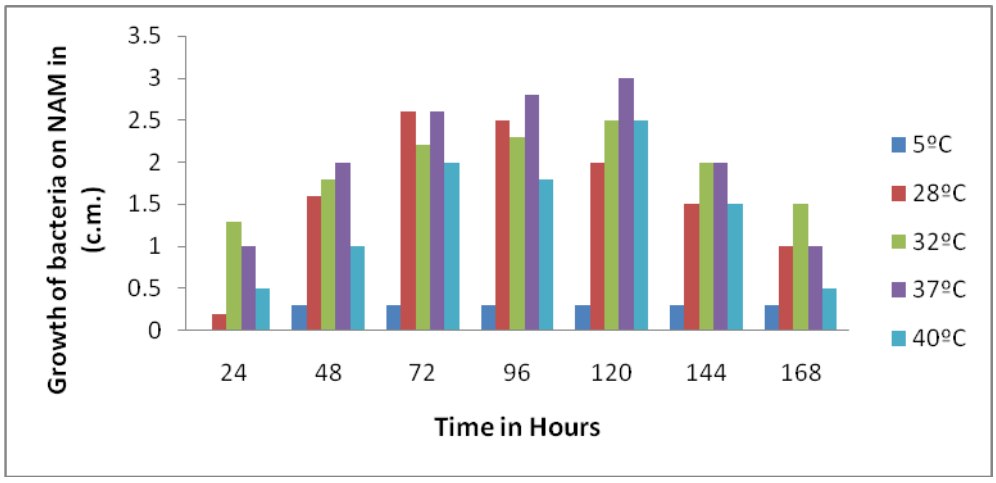


Fig.2 Impact of different pH on the melanoidin decolourization by selected bacterial strain

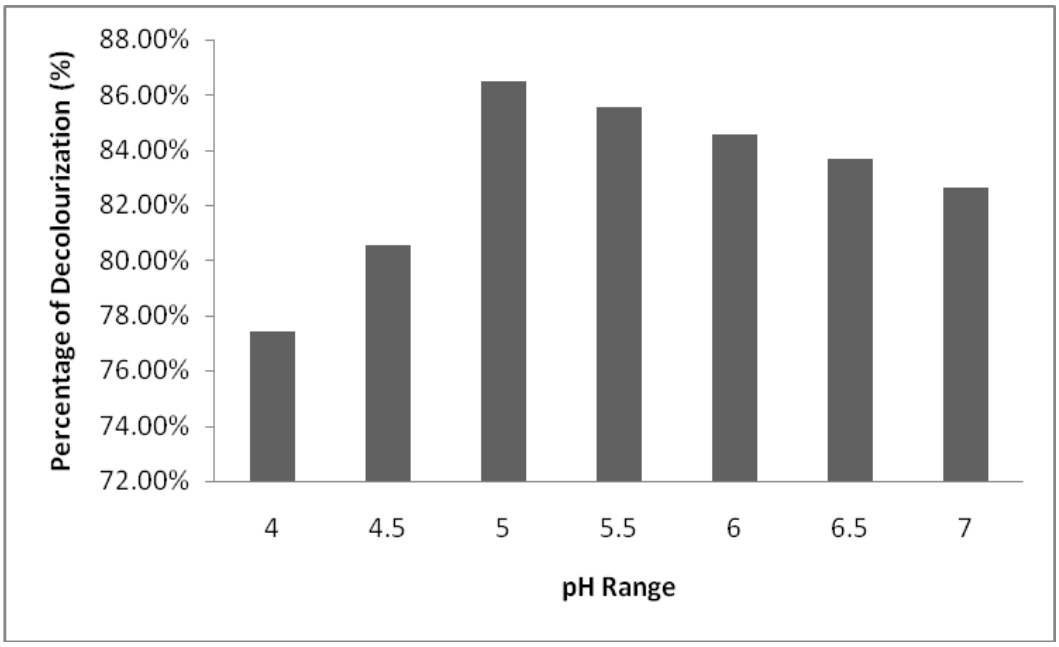


Fig.3 Impact of various carbon sources on the melanoidin decolourization by selected bacterial strain.

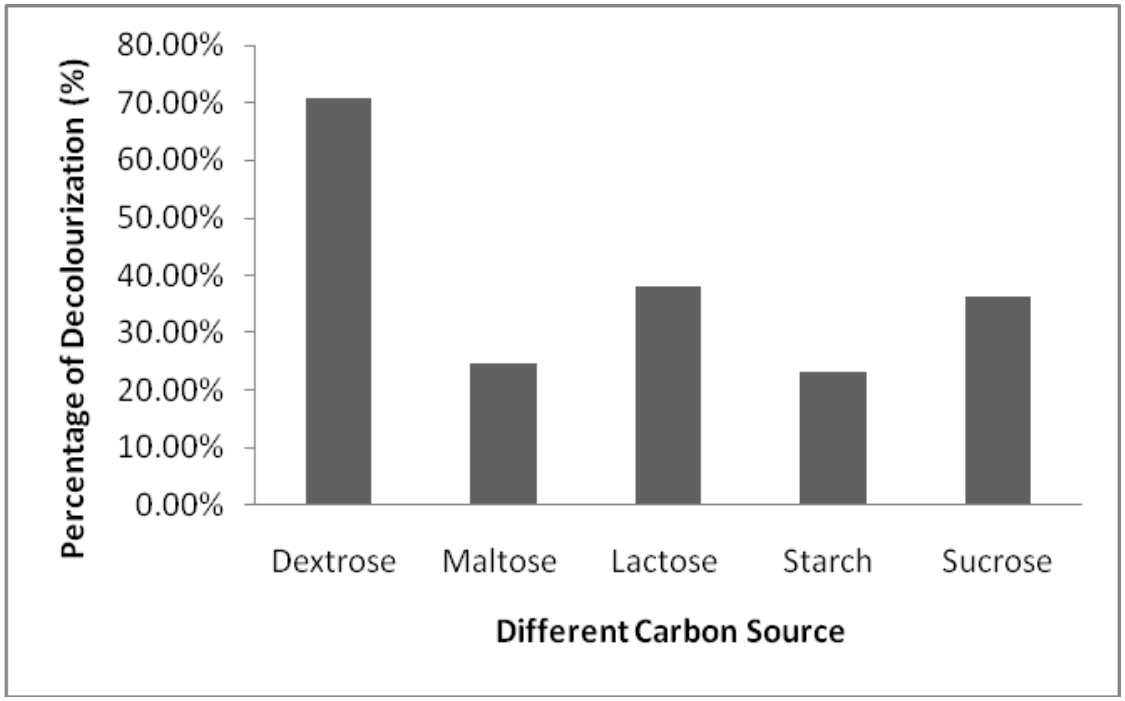


Fig.4 Impact of Various concentrations of dextrose on melanoidin decolourization by selected bacterial strain

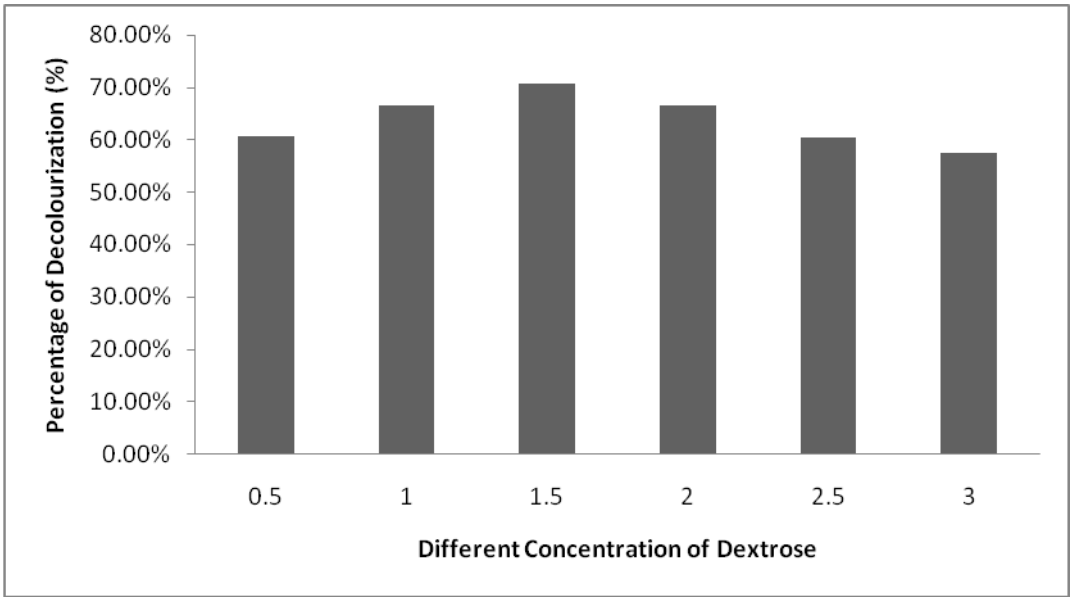


Fig.5 Impact of various nitrogen sources on the melanoidin decolourization by selected bacterial strain.

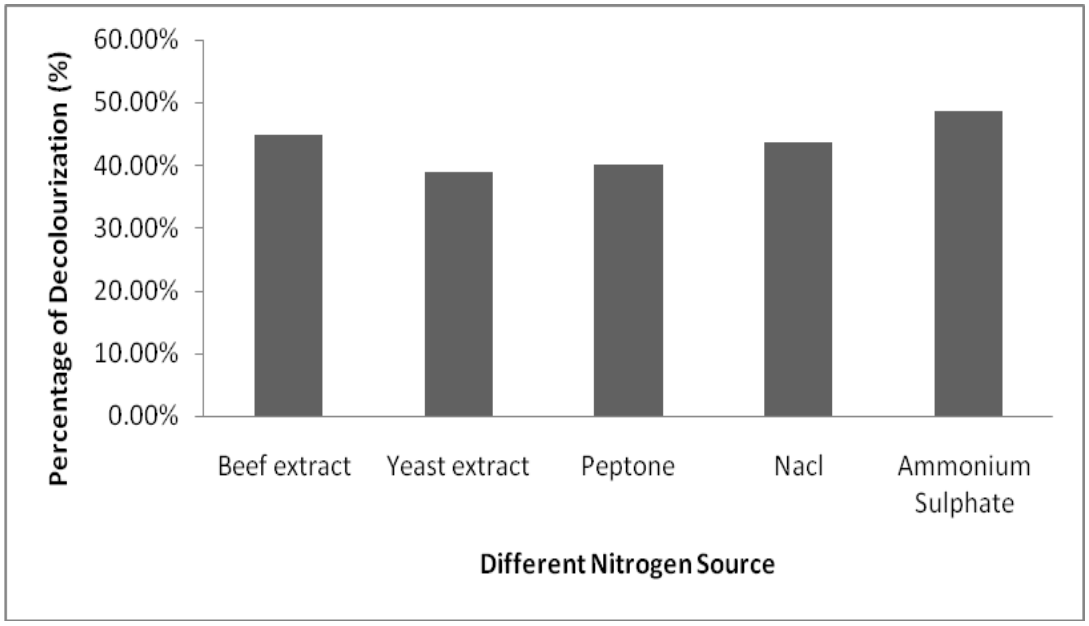
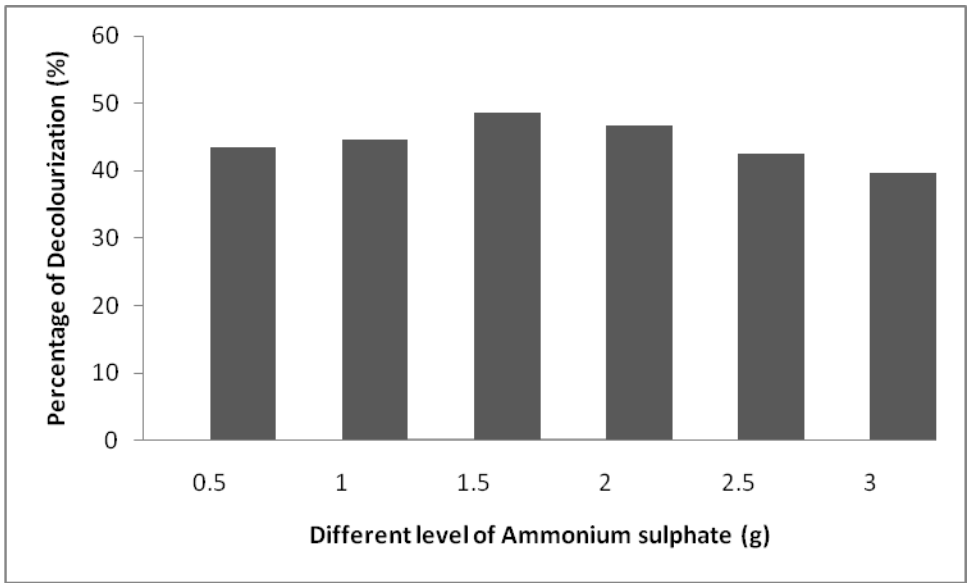


Fig.6 Impact of different concentration of ammonium sulphate on melanoidin decolourization by selected bacterial strain



Impact of Various Nitrogen Sources on Decolourization of the Melanoidin by Selected Bacterial Strain

Requirement of nitrogen compounds for initiation of microbial growth is a well-known fact. The influence of nitrogen on decolourization was studied by supplementing distillery effluent with different nitrogen sources. Different nitrogen sources say- Beef extract, Peptone, Yeast extract, Ammonium sulphate and Sodium chloride were individually tested for decolourization of effluent by the bacterial strain. The concentration of the nitrogen sources was taken 1.5 gm. From the given data it was observed that maximum decolourization was observed in ammonium sulphate (48.58%) followed by beef extract (44.80%).

Impact of Different Concentrations of Ammonium sulphate on Decolourization of Melanoidin by Selected Bacterial Strain

Different levels of Ammonium sulphate (0.5gm, 1.0gm, 1.5gm, 2.0gm, 2.5gm and 3.0gm) were supplemented to distillery effluent. From the result it can be observed that maximum decolourization was noted at 1.5g of the Ammonium sulphate concentration. Further increase or decrease in concentration did not show much decolourization of the distillery effluent.

According to established procedures, the effluent was characterised for colour, pH, temperatures, COD, BOD, phosphorus contents, nitrogen content, carbon content, and total solids (Ravikumar, 2015).

According to the study's findings, the culture is unable to survive high temperatures. According to pH optimization experiments, neutral pH was better for decolorization than alkaline or acidic pH. The findings now match those that were previously published (Singh *et al.*, 2007). The biodegradation of used wash has been researched by several academics (Ravikumar, 2015; Naik *et al.*, 2010; Singh *et al.*, 2007). By utilising *Cladosporium cladosporioides*, Ravikumar (2015) observed 72.3% decolorization of wasted wash in only 5 days (Ravikumar, 2015).

According to the ongoing research, microbial removal of distilleries discharge may be a convenient, economical, and environmentally friendly method of removing the pigment melanoidin.

Acknowledgement

The authors would like to extend their gratitude to the administration of Dr. Rammanohar Lohia Avadh University for enabling them to carry out the study by providing the required resources.

Conflict of Interest

There is no conflict of interest of any kind among the authors.

Funding Agency

Ranjan Singh would like to express his gratitude to the Department of Higher Education, Government of Uttar Pradesh, Lucknow, Uttar Pradesh, India (47-2021/606/77-4-2021-4/56/2020), which funded the "Research and Development of State Universities of Uttar Pradesh" project. In particular, he would like to thank the "Research and Development of State Universities of Uttar Pradesh" plan. The Department of Higher Education, Government of Uttar Pradesh, Lucknow, Uttar Pradesh, India, has provided RajeevaGaur with financial assistance under the programme "Research and Development of State Universities of Uttar Pradesh (108/2021/2588/77-4-2021-4(28)/2021), for which RajeevaGaur would like to express their gratitude.

References

Ravikumar R. Effect of transport phenomena of *Cladosporium cladosporioides* on decolorization and chemical oxygen demand of distillery spent. Int. J. Environ. Sci. Technol. 12; 2015: 1581–1590. https://doi.org/10.1007/s13762-014-0520-4

Naik N, Jagadeesh K S, Noolvi M N. Enhanced degradation of melanoidin and caramel in biomethanated distillery spent wash by microorganisms isolated from mangroves. *Iranica Journal of Energy & Environment*. 2010; 1 (4): 347-351.

Singh K D, Sharma S, Dwivedi A, Pandey P, Thakur R L, Kumar V. Microbial decolorization and bioremediation of melanoidin containing molasses spent wash. *Journal of Environmental Biology*. 2007; 28(3): 675-677.

Prescott S C, Dunn, C. G. 'Industrial microbiology 2nd Ed, published by Mm–Graw hill Book company New York, U. S. A. and Rao M. N. and Datta A. K. 'waste water treatment in rational methods of Design and industrial process 1979.

Chavan M N, Kulkarni M V, Zope V P, Mahulikar P P. Microbial degradation of melanoidins in distillery spent wash by an indigenous isolate. *Indian journal of Biotechnology* 2006;416–421.

Santal A R, Singh N P, Saharan B S. A novel application of *Paracoccus pantotrophus* for the decolorization of melanoidins from distillery effluent under static conditions. *Journal of environmental management*. 2016;169:78-83. https://doi.org/10.1016/j.jenvman.2015.12.016

Gaikwad B G, Pisal S H. Biodegradation of Spent Wash using Bacteria and Yeast. *Screening*. 2016;3077:8-66.

Khandekar Y S, Shinkar N P. Distillery spent wash biological treatment techniques: A Review. International *Journal of Innovative Research in Advanced engineering (IJIRAE)* 2020;7:252-8.

Shinde A B and Nakade D B. Decolourization of Distillery Spent Wash by Bacillus coagulans ABS012. 2021. https://doi.org/10.15515/abr.0976-4585.12.1.90100

Duraisamy T, Selvam R, Muniraj S, Mubarakali H, Muthunarayanan V. Deportation of melanoidin in distillery spent wash using bacterial consortia-confirms through structural changes and Ao-Eb fluorescence. *International Journal of Development Research*. 2017;7(09):15392-400.

How to cite this article:

Ajad Patel, Ranjan Singh, Abhishek Vashishtha, Soni Tiwari, Prabhash Kumar Pandey, Vijay Kumar Shukla, Laxmi Kant Pandey, Femina Sobin, Tanim Arpit Singh, Trashi Agrah Singh, Neeraj Khare, Shipra Singh, Rajeeva Gaur and Mahendra Kumar Gupta. 2023. Decolourization of Spent Wash using Bacteria *Pseudomonas putida*. *Int.J.Curr.Microbiol.App.Sci.* 12(10): 63-73.
doi: https://doi.org/10.20546/ijcmas.2023.1210.008