Bioremediation Studies on Melanoidin Containing Distillery Spent Wash by Using *Leuconostoc mesenteroides*

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Abstract Melanoidins are the natural condensation products present in distillery spent wash. It is the result of Millard reaction which occurs between glucose and glycerine. From environmental aspects, melanoidins are very important because of the complex structure and caliginous colour and objectionable odour, which can cause damage to the surroundings. All these characteristics of melanoidin modify the photosynthetic activity of plants, reduce the dissolved oxygen of aquatic ecosystem, soil fertility is affected to a large extent, and it also slows down the sprouting of seeds. Bioremediation is an environmentally friendly technology for the treatment of chemical and hazardous waste. Bacteria from the nearby contaminated soil have a high potential to convert complex bioorganic substances to simpler absorbable form. With reference to this utilization of bacteria, *Leuconostoc mesenteroides* is an economical technology for the treatment of contaminated water having melanoidin. Thus, the use of this novel microorganism in the field of environmental biotechnology will be an effective way to solve the biggest problem of water and soil pollution.

Keywords Bioremediation, Melanoidin, *Leuconostoc mesenteroides*, Distillery Spent Wash (DSW)

1. Introduction

Today with the advent of industrialization, urbanization, the exploitation of resources is also reaching its maximum level. Population explosion resulted in magnification of industrial sectors which also results in contamination of water, air and soil.

Preventing pollution is somewhat difficult, but by following some eco-friendly technology, we can control the pollution [1, 2]. One of the developing industries is the distillery unit. DSW (Distillery spent wash) is the leftover that is generated while producing the alcohol. As per the previous researches, molasses cane dependent alcohol industries produce fifteen liters of spent wash from one liter of alcohol. According to the reports, there are a total of two hundred and twelve alcohol producing industries in India which produce around thirty billion liters of spent wash per year [3]. Due to Millard’s reaction in which
Amino carbonyl reaction produces melanoidin which gives dusty colour to the spent wash [4].

Three concepts of decolourisation, degradation and mineralisation of coloured effluents are often talked about vaguely and misused. Decolourisation is the minimization of the colour from the effluent without transformation of the spent wash, thus it doesn’t ensure the degradation of spent wash. In effluent degradation, the big complex coloured substances are converted to simpler absorbable molecules.

In Mineralization, carbon components are transformed to inorganic substances, as NO₃, CO₂ and H₂O. Bioremediation consists of biodegradation and biotransformation, with the expectations of removing harmful toxicants from the environment.

As melanoidin has various challenging properties like solubility, volatility & the complex structure, the modified analytical tools must be selected to check the decolourisation and degradation mechanisms.

So in the present research studies, some advanced analytical tools like spectrophotometry, high performance liquid chromatography and Fourier transform infrared resonance were followed.

The structural complexity of the melanoidin from spent wash and its transformations process is still not well understood. The identification of such unknown metabolite which is a very difficult task and therefore a combination of techniques had been used to tackle the problem.

According to previous research studies, UV/VIS Spectrometry is very commonly being implemented to give evidence of distillery spent wash degradation and decolourisation. Fourier Transform Infrared Resonance (FTIR) is another tool to follow the melanoidin degradation mechanism [5].

Chemical and physical methods of degradation of melanoidin containing distillery spent wash are reported by various researchers, but the use of microorganism as *Leuconostoc mesenteroides* in the present research work is the productive technique. We have followed UV/VIS spectrometry, FTIR and HPLC for the analysis of decolourisation as well as degradation. In the first step of spectrophotometric analysis the melanoidin degradation activity was assayed by measuring the decrease in absorbance at 450nm against the initial absorbance at the same wavelength as the absorbance maxima for the prepared standard melanoidin was 450nm. Next to it the tools as FTIR and HPLC were implemented.

### Characterization of the melanoidin containing distillery spent wash

The characterization of distillery spent wash by estimating the various parameters viz. pH, BOD, COD, total dissolved solids, total sugars, magnesium, iron, sulfates, chlorides, phosphorus and oil & grease were estimated as per the standard protocols given by APHA, USA [6].

### Standard melanoidin preparation

For the confirmation of maximum absorbance, standard melanoidin is synthesized in the lab by heating 180.16g/l glucose with 0.5 Molar of glycine amino acid at 90°C and pH was maintained at - 5.5 for 6 hr [7]. Hot air oven was used to maintain a high temperature. The maximum absorption of prepared melanoidin was verified at 450 nm by making use of double-beam Spectrophotometer and as a result standard curve was obtained. The absorption maxima for the collected spent wash was the same as for standard melanoidin. So the next bioremediation studies were carried out at 450 nm.

### Decolourisation experiment

After optimization studies of media and spent wash, loopful pure culture of *Leuconostoc mesenterioides* was used and inoculated in the flask of 40% spent wash [8,11]. The decolourised spent wash was centrifuged at 7000 rpm for twenty minutes, the supernatant after filtration by micronfilter was used for the UV/VIS analysis, FTIR, and HPLC while following these techniques 40% spent wash were used as the control.

### Identification of degraded melanoidin metabolites by UV/VIS spectrometry

Decolourised and degraded melanoidin containing 40% spent wash was used in this study and monitored using UV/VIS spectrometer. Double Beam spectrometer model Shimadzu 1700 Pharmaspec with 200nm to 800nm wavelength was used.

### Identification of metabolites by FTIR

Dry standard melanoidin was used as the control while all decolourised media by the *Leuconostoc mesenterioides* were dried in the hot air oven and used for the analytical study. As FTIR can be followed for dry or crystalline materials only.

Shimadzu-8400 S FTIR spectrophotometer (Shimadzu Tokyo, Japan) was used and the sample was analyzed in the region of 400-4700 cm⁻¹ mid IR with the speed of 25 scans on the hexagonal demountable cell.

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### 2. Materials and Methodology

#### Isolation and characterization of microorganism

The isolation of melanoidin degrading organism was isolated by enriching the spent wash and soil samples collected from the local distillery units [13]. The most efficient organism was characterized by the VITEC system ver 2.0.
Identification of metabolites by HPLC

HPLC analysis was carried out at the Instrumentation Lab of K.T.H.M. College, Nashik, Maharashtra. Decolourisation and degradation of melanoidin containing distillery spent wash by *Leuconostoc mesenteroides* was analyzed by HPLC (Shimadzu). Nearly 10ml of control and degraded spent wash samples were centrifuged and filtered through the 0.45µm membrane millipore filter. After filtration, the filtrate was checked with mobile phases with acetonitrile and methanol (45:55) (HPLC grade) containing 1ml of acetic acid and 0.5gm sodium acetate 3H2O with pH around 5. The standard flow rate of the above system was 0.8ml/min. Wokosil C-18, 5µm SGE, 250x4.6 mm stainless steel was the stationary phase of the column, and with the ULTRA VIOLET detector (UV-1580 SPD-10A single) resultant peak was then analyzed.

Changes in the peak length and retention time of all observations were the clues for the degradation of melanoidin from the spent wash.

3. Results and Discussion

Isolation and characterization of microorganism

From the various nearby (Distillery) soil samples fifteen different organisms firstly were isolated and after their screening for maximum melanoidin degradation the most efficient organism was characterized and identified as *Leuconostoc mesenteroides* by the VITEC system ver.2.0 at the Bac-test laboratory, Nasik, and shown in the following fig. 1 [9,14,15,17].

![Figure 1. VITECH Chart for Leconostoc mesenteroides](image-url)
Table 1. Characterization of distillery spent wash before and after use of *Leuconostoc mesenteroides*

| Sr No | Parameters         | Before treatment | After treatment |
|-------|--------------------|------------------|-----------------|
| 1     | Colour             | Dark Brown       | Light Brown     |
| 2     | Odour              | Strong pungent   | Mild            |
| 3     | PH                 | 4.3              | 6.7             |
| 4     | BOD (mg/L)         | 60,540           | 38,370          |
| 5     | COD (mg/L)         | 95,680           | 49,500          |
| 6     | Total sugar (mg/L) | 12,300 - 90,000  | 800 – 1,400     |
| 7     | Total dissolved solids | 7,800       | 1,800           |
| 8     | Iron (mg/L)        | 124              | 90              |
| 9     | Magnesium (mg/L)   | 2,550            | 440             |
| 10    | Sulphates (mg/L)   | 980              | 860             |
| 11    | Free chlorides (mg/L) | 7,000          | 550             |
| 12    | Phosphorus (mg/L)  | 4850             | 600             |
| 13    | Oil and Grease (mg/L) | 174            | 162             |

Characterization of the distillery spent wash

The various physicochemical parameters of distillery spent wash are characterized before and after the microbial treatment with *Leuconostoc mesenteroides* are summarized in table 1 [3].

After treatment, physicochemical analysis demonstrated a considerable decrease in many parameters as mentioned in the above table 1. Thus by following such technology, the treated spent wash can be discharged in river water.

Standard melanoidin preparation

The dark brown coloured melanoidin was successfully synthesized in the lab by heating the mixture of glucose and glycine. The absorption maxima for both standard and synthesized melanoidin were found at 450 nm. The colour and consistency of the synthesized melanoidin and collected distillery spent wash are shown in fig.2 and fig.3 respectively.

![Standard melanoidin](image1)

![Distillery spent wash](image2)

Decolourisation experiment

![40% DSW Before Treatment](image3)
The media containing 40% of distillery spent wash were treated with bacterial isolate *Leuconostoc mesenteroides*. For its decolourisation under static condition showed up to 64.5% reduction in the colour [16]. The flasks showing colour reduction are given in fig.4 and fig.5 respectively.

**Identification of metabolites by UV/VIS Spectrometry**

The media containing 40% spent wash, treated with *Leuconostoc mesenteroides*, centrifuged and filtered were subjected to spectrophotometric analysis, the disappearance of major melanoidin peak and appearance of new peaks of metabolites are shown in fig.6.

The comparative UV/VIS scan of the melanoidin containing spent wash showed the reduction in the absorbance of the decolourised sample. The changes in the peaks and appearance of new peaks indicate the decrease in melanoidin concentration by the organism.

**Figure 5.** 40% DSW After treatment

**Figure 6.** UV-VIS. Spectral analysis of Control 40% DSW [Above] and 40% degraded DSW by *Leuconostoc mesenteroides* [Below]
The minimization in optical density of the melanoidin containing spent wash at their λ max (330 nm and 283 nm respectively) and the formation of new peaks in the IR spectra, compared to control sample, were the clues for their degradation. Cleavage would generate hydroxyl groups of 3400 bands. With this belief, the intensity of 3400 bands showed a marked increase in treated samples compared to the control sample. Treated spent wash IR spectra was compared to the IR spectra of the standard melanoidin. Changes in both peaks reveal that there is the transformation of the polymeric compounds present in the spent wash into the simpler forms [5].

The media containing 40% spent wash, treated with *Leuconostoc mesenteroides*, centrifuged and filtered were subjected to FTIR analysis and are shown in fig.7. From the analysis report of HPLC as in Fig. 8(a) and Fig. 8(b), the area, height of the peak and retention time, of the melanoidin containing spent wash, before and after the treatment confirms the biodegradation of melanoidin of the spent wash [10]. A vital peak at the retention time of treated spent wash, by *Leuconostoc mesenteroides* was less as compared to untreated spent wash. Thus it indicates the ability of *Leuconostoc mesenteroides* to decolourise as well as to degrade the melanoidin containing spent wash. Differences in the length of the peaks confirm the degradation of the spent wash [3].
**Figure 8a.** HPLC analysis of 40% DSW Control

**Figure 8b.** HPLC analysis of 40% DSW degraded by *Leuconostoc mesenteroides*. 
4. Conclusions

Various analytical tools were used in this study, which were successfully monitored for the decolourisation of 40% melanoidin containing distillery spent wash by *Leuconostoc mesenteroides*. UV/VIS analysis confirmed degradation of heterogeneous polymers of melanoidin containing spent wash. FTIR result confirmed the mineralization of melanoidin intermediates of the spent wash, while HPLC results indicate that *Leuconostoc mesenteroides* degrade the melanoidin to its corresponding intermediates. These analytical studies conclusively suggested the crucial role of *Leuconostoc mesenteroides* which work for the eco-friendly biodegradation of 40% melanoidin containing spent wash.

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