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MICROBIAL DEGRADATION OF DISTILLERY EFFLUENT BY USING MIXED CONSORTIUM

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ABSTRACT

Effluent originating from distilleries which contain large amount of dark brown colored wastewater called Molasses Spent Wash (MSW). Dark brown colour of MSW is due to the presence of Melanoidin pigment. It generally produced by 'Millard' reaction and it is recalcitrant polymer. If dispose to untreated posses a great threat to environment. Melanodin was degraded or decolourise by using different microbial species. Microbial decolourization and degradation on the basis of (COD) chemical oxygen demand and spectrophotometer analysis were found to be dependent on the specific carbon and nitrogen source. The collection of indigenous bacterial cultures was performed to estimate its ability to degrade harmful pollutants present in molasses spent wash (MSW) and to understand its degradation potential through natural attenuation for which different bacterial species (*Pseudomonas aeruginosa, Proteus vulgaris,* Two *Bacillus isolates)* was utilized. From the following results 1.2% glucose/peptone concentrations were considered to be the optimized source for carbon and nitrogen for the organisms. Further, the degradation potential of the consortia (*Pseudomonas aeruginosa, Proteus vulgaris, and* Two *Bacillus isolates*) in different concentrations of MSW along with its application on potted Soyabean plant was studied.

Keywords: Spent wash, physicochemical analysis, Decolourization, Field Trials

INTRODUCTION

At present, there are 285 distilleries in India that are producing 2.7 billion liters of alcohol and generating 40 billion liters of waste water annually **[1]**. Distillery spent wash is the unwanted residual liquid waste generated during alcohol production in distilleries which has unpleasant odor. On an average 8–15L of effluent is generated for every liter of alcohol produced **[2, 3]**. 'Distillery spent wash' is recalcitrant in nature due to presence of the brown polymers, "Melanoidin", which are formed by Maillard amino carbonyl reaction **[4]**. This compound has antioxidant properties **[5]**. Spent wash disposal into the environment is hazardous and has high pollution potential. High COD, Total Nitrogen and Total Phosphate content of the effluent may result in eutrophication of natural water bodies **[5, 6]**. The highly coloured components of the spent wash reduce sunlight penetration in rivers, lakes or lagoons which in turn decrease both photosynthetic activity and dissolved oxygen concentration affecting aquatic life.

Disposal of untreated distillery spent wash on land is equally hazardous to the vegetation. It is reported to reduce soil alkalinity and manganese availability, thus inhibiting seed germination **[7]**. Knowledge on physicochemical characters of distillery spent wash waste water is essential for design operation, collection and treatment as well as disposal facilities for the effective management of environmental qualities. The use of mixed consortia *(Pseudomonas aeruginosa, Proteus vulgaris,* and Two *Bacillus isolates*) approach to remove Melanoidin is ecofriendly and cost effective. After removal of Melanoidin from spent wash, its best application is as bio-liquid fertilizer for sustainable agriculture. The use of treated spent wash as a liquid biofertilizer can also be cost effective and technically feasible.

MATERIAL AND METHODS

Sample collections

Sample of biomethanated distillery effluent was collected from Bhagwati Shakari Sakhar Karkhana Limited (BSSKL), Patas, Pune, and Maharashtra, India. The contaminated soil was collected from the site nearby the distillery unit. The soil was collected by scrapping the top layer of soil and subsurface soil and packed in an air tight sterile bag **[7, 8]**.

(2.1) Collection of sample: Shree Bhogawati Sahakari Sakhar Karkhana , Pvt. Ltd. Shahunagar , Parite, Kolhapur.

Sampling site

Sampling site Map

Isolation and Identification of organism by enrichment technique

Microorganisms screening was done by enrichment method **[9]**. The tubes showing decolorization were subsequently sub cultured four times and isolation of microbial culture was carried out on minimal salt glucose medium by spread plate technique. The pure culture of different microbial isolates S1-S5 were maintained on minimal salt glucose agar medium containing 5% spent wash **[10]**.

Distillery Spent Wash (DSW)

The molasses spent wash was collected aseptically from Bhima Shakari Sakhar Karkhana Limited (BSSKL), Patas, Pune, and Maharashtra, India.

The spent wash was centrifuged at 10,000 rpm for 15 min before use to remove the suspended solids and stored at 4°C **[11]**.

The stored distillery spent wash was filtered through (Whatman No: 1) filter paper and was diluted with distilled water. The analysis of different physico-chemical parameters like color, odour, pH, Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), total sugars, Total Dissolved Solids (TDS), sulphates, phosphorous and calcium were analysed for employing standard

methods for examination of water and wastewater **[12]**, result is shown in **Table 1**.

Isolation, screening and identification of Melanoidin decolorizing bacteria

Melanoidin decolorizing bacteria isolated from soil sample collected from Bhima Shakari Sakhar Karkhana Limited (BSSKL), Patas, Pune, and Maharashtra, India, was grown on minimal salt glucose agar medium containing 5% spent wash medium for 24-48 h incubation. Culture medium consisted of 0.01%, KH_2PO_4; 0.05%, $MgSO_4.12H_2O$; 0.5%, glucose and 0.1%, yeast extract with 3.5 OD effluents and the initial pH was adjusted to 6.0. In order to isolate molasses-decolorizing bacteria, 1g of soil was serially dilution upto 10^{-5} -10^{-6} and placed in Petri-plates along with the basal agar medium. The plates were subsequently incubated for 24-48 h at 35±2°C. After 24-48 h of incubation decolorization effect was seen visually. The isolates showing higher decolorization of the melanoidin were selected for further studies, maintained on the same medium at 4°C in slants and sub-cultured after 15 days.

Isolation and identification of microorganisms till genus level by 16s rRNA sequencing method from IISER Pune.

Inoculum preparation

Cell suspension was prepared by inoculating 1 mL of 24 h grown culture in 50 mL basal broth and then incubated at 35°C for 24 h to achieve active exponential phase of culture consisting 5×10^6 cfu/mL transferred into the flask and incubated in static condition. Quantitative decolorization value was determined on the basis of OD at 475 nm against the blank by UV-visible Spectrophotometer.

Decolourization assay of the spent wash

The melanoidin decolorizing bacterial isolates were inoculated in the basal broth medium and after incubation; broth was centrifuged at 10,000 rpm for 10 min. The supernatant of the centrifuged sample will read at absorbance maximum (Amax) of the melanoidin i.e., 475 nm using spectrophotometer **[13]**.

The decolorization yield will be expressed as the decrease in the absorbance at 475 nm against initial absorbance at the same wavelength. Uninoculated medium will serve as control. The entire assay was performed in triplicate and compared with control.

The decolourization efficiency of the different isolates will be expressed as per following equation:

Decolourization (%) = I - F / I

Where: I = Initial absorbance (Control) and F = Absorbance of decolorized medium broth.

Optimization of culture conditions for decolourization

Selection of physical parameters for melanoidin decolorization: The basal medium for melanoidin decolorization with different temperature viz. 35, 40, 45, 50 and 55°C and incubation period viz. 8, 16, 24, 32 and 40 h were used for the melanoidin decolorization. The initial pH (6.0) was varied in the medium by adding either 1N HCl or 1N NaOH as required. The basal medium was then inoculated with 0.5% (v/v) inoculum of bacterial isolates having $5x10^6$cfu/mL population respectively and incubated at different pH viz. 5.0, 5.5, 6.0, 6.5 and 7.0 for optimization of melanoidin decolorization.

Selection of nutritional parameters for melanoidin decolorization

Various carbon sources viz. glucose, fructose, sucrose and lactose at 0.5% (w/v) were individually added in the basal medium and inoculated with 0.5% (v/v) of bacterial cultures separately with their respective optimized pH, temperature then incubated for 24h for decolorization. The best source ofsugar will further optimized in different concentration viz. 0.1, 0.2, 0.3, 0.4, 0.5 and 0.6% (w/v) for melanoidin decolorization. In another experiment, different organic and inorganic nitrogen sources viz. beef extract, yeast extract, peptone, ammonium sulphate and ammonium nitrate were individually added into the basal medium at 0.5% (w/v). Active culture of individual bacteria was inoculated with 0.5% (v/v) inoculum having 5×10^6cfu/mL. The best source of nitrogen will further optimized in different concentration viz. 0.1, 0.2, 0.3, 0.4 and 0.5% (w/v) for melanoidin decolorization.

Statistical analysis

All the experiments were carried out in triplicates and the results are presented as the mean of three independent observations. Standard deviation for each experimental result was calculated using Microsoft Excel.

RESULT

Physicochemical analysis: Table 1 show physical and chemical properties of spent wash sample obtained from sugar industries as.

Isolation, screening and identification of melanoidin decolorizing bacteria (Figure 1, 2).

Decolourization assay of the spent wash (Figure 3).

% COD reduction assay of the spent wash at different concentration (Figure 4).

HPLC result of spent wash (Figure 5).

HPLC profile of melanoidin degradation study of spent wash by mixed consortium (Table 2).

Table 1: Shows Physicochemical parameters of Boimethanated spent wash before treatment and after treatment

Parameters	Before Treatment	After Treatment
pH	4.2	6.8-7.2
Color	Brown	Pale brown
Odour	Unpleasant	Odorless
Electrical conductivity	22.4/ ds/m^{-1}	0.986 dsm^{-1}
Total solid	186400 mg/lit	64246 mg/lit
Total suspended solid	92800 mg/lit	40568 mg/lit
Total dissolved solid	93600 mg/lit	23678 mg/lit
Total hardness	107.37 mg/lit	76.9 mg/lit
Chemical oxygen demand	2688 mg/lit	1317 mg/lit
Biological oxygen demand	-	1317 mg/lit
Nitrogen	883.5 mg/lit	784.5 mg/lit
Chloride	3500 Cl mg/lit	200 Cl – mg/lit
Sulphate	75880 mg/lit	34670 mg/lit
Phosphorous	26600 mg/lit	12788 mg/lit

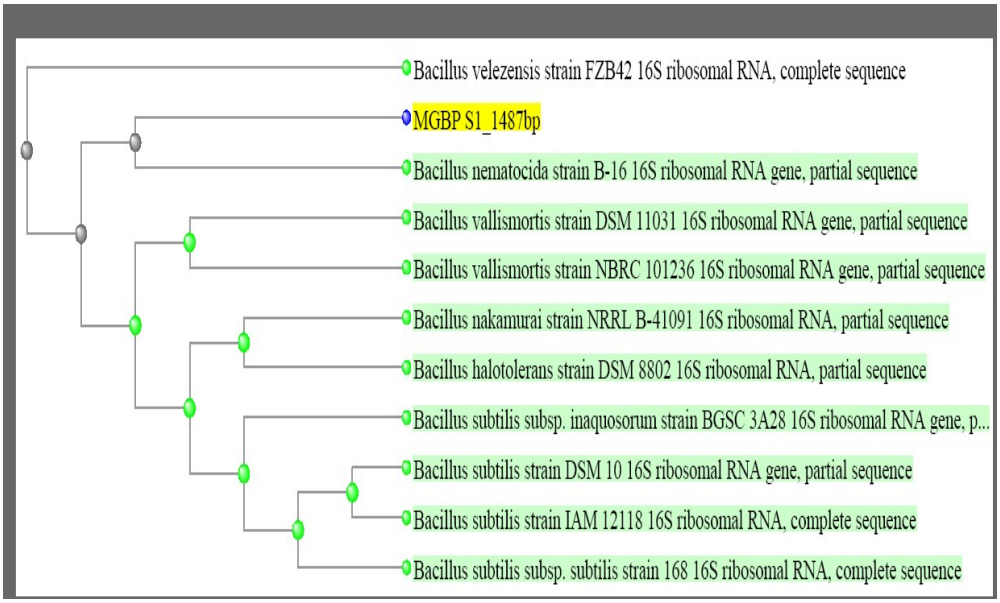

Figure 1: Phylogenetic position based on 16SrRNA gene sequence analysis of bacteria Bacillus species

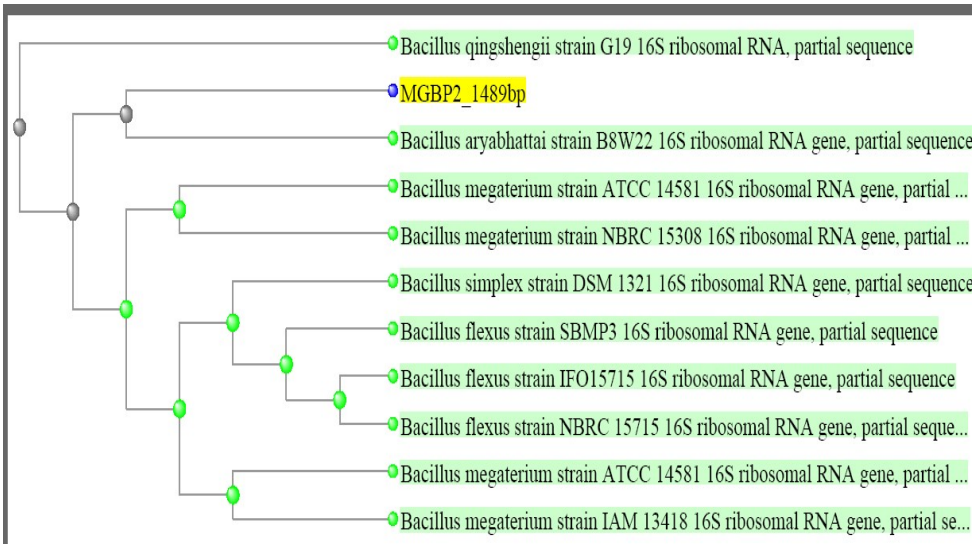

Figure 2: Phylogenetic position based on 16SrRNA gene sequence analysis of bacteria Bacillus species

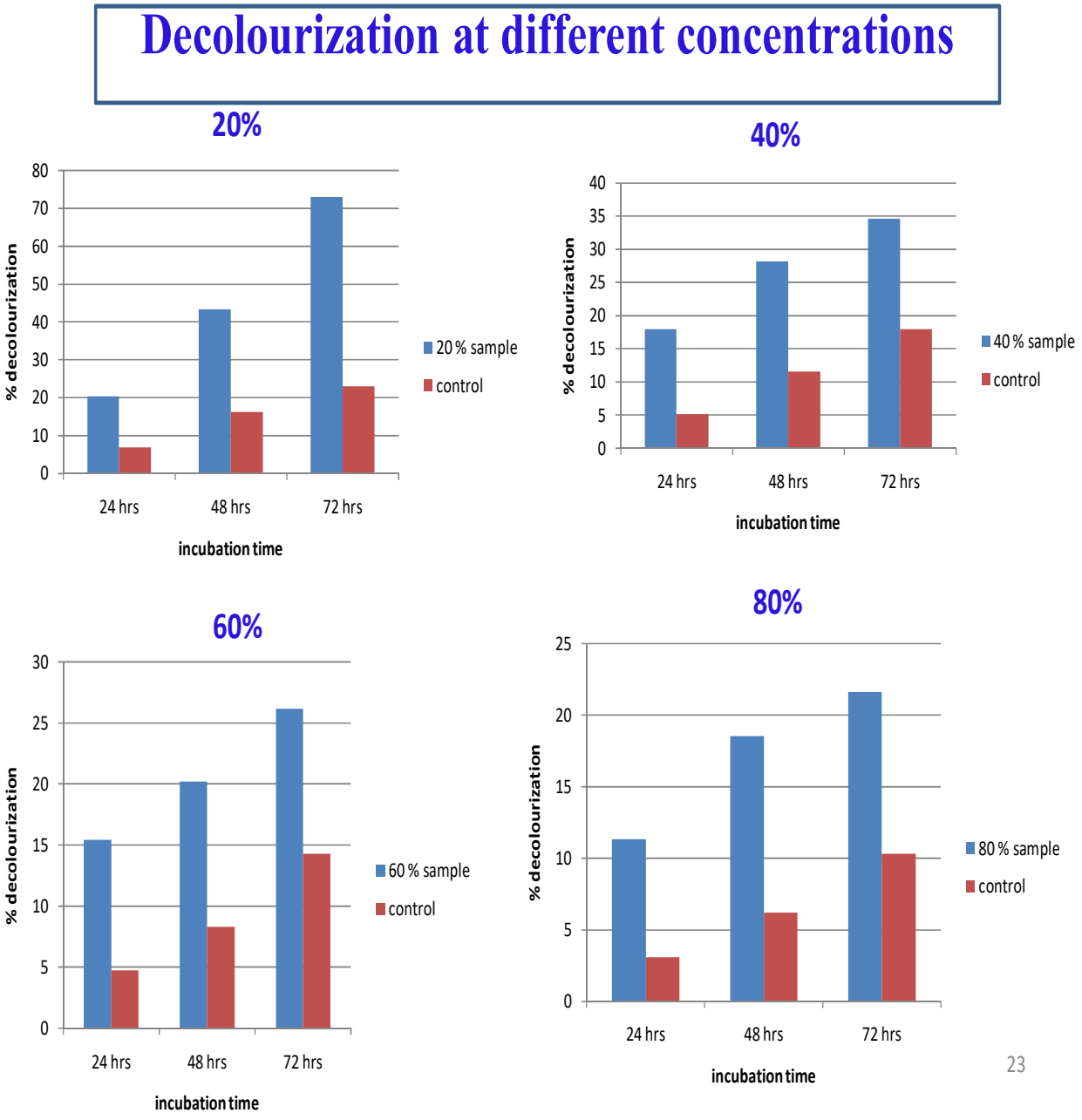

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Figure 3: Decolourization assay of the spent wash at different concentration

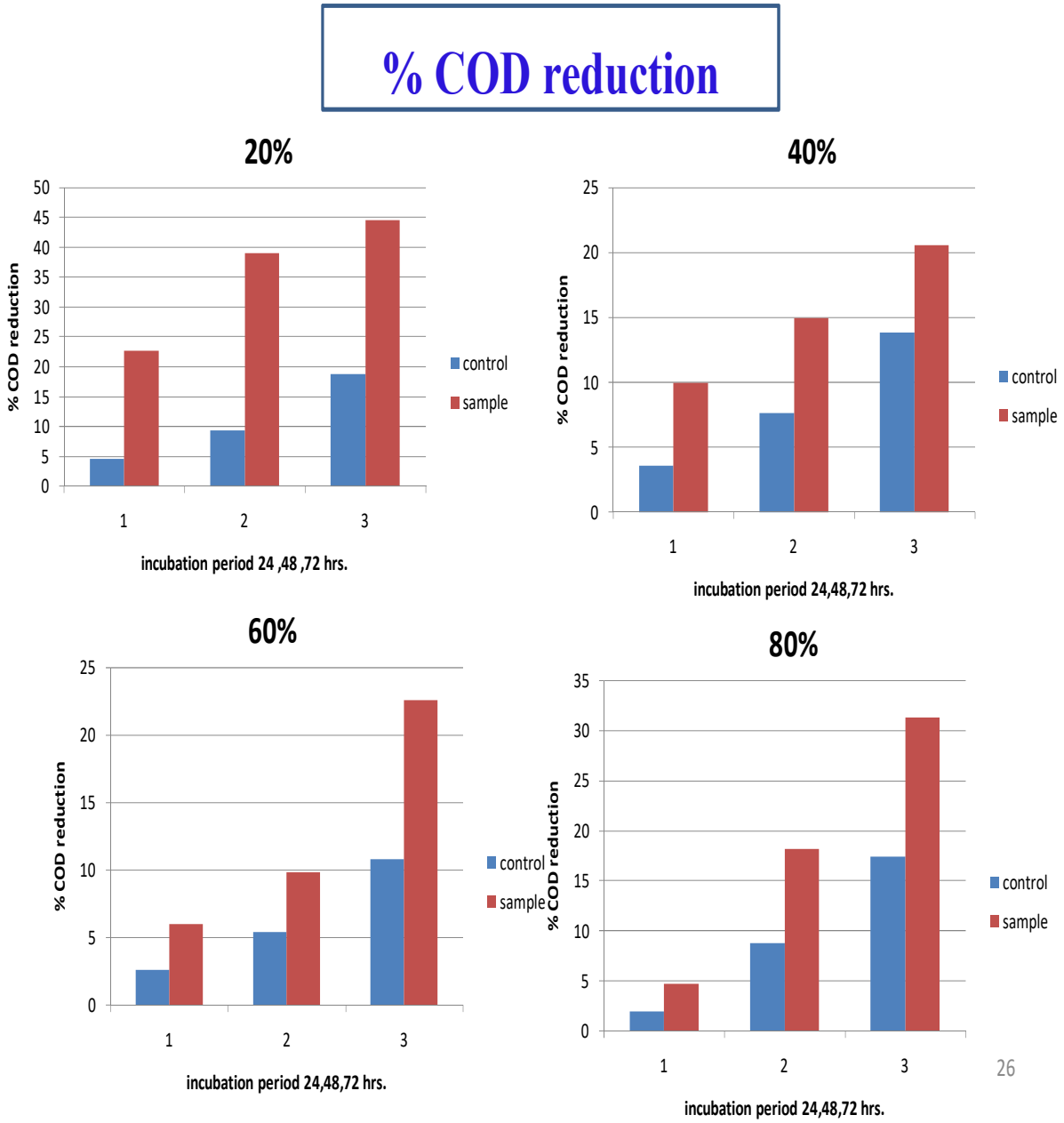

Figure 4: % COD reduction assay of the spent wash at different concentration

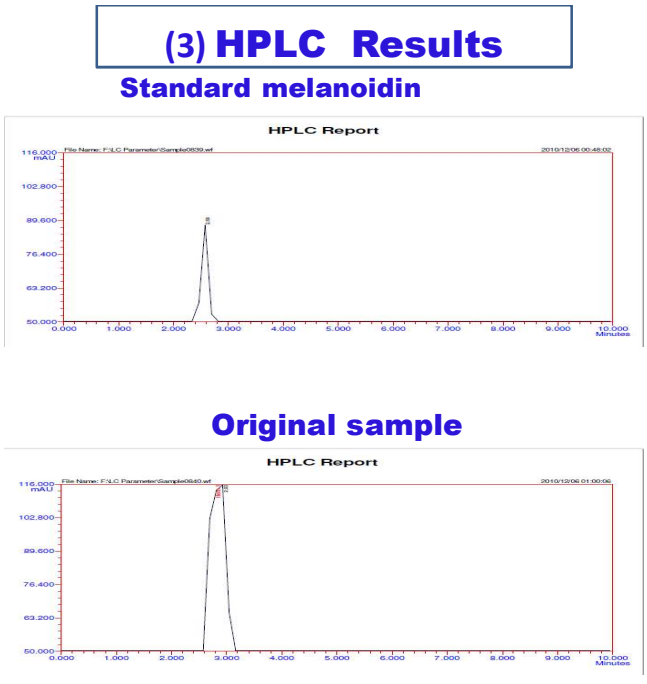

Figure 5: HPLC result of spent wash

Table 2: HPLC profile of melanoidin degradation study of spent wash by mixed consortium

Sample	Retention Time	Area	Height	Concentration
Standard	2.575	8881	1177926.8	100.000
Original sample	2.925	11810	301691.6	100.000
24 hrs. sample	2.805	33868	958706.4	100.000
48 hrs. sample	2.925	33091	718992.6	100.000
7 2hrs. sample	2.692	26814	597214.5	99.422

DISCUSSION

Disposal of waste could pose a serious health hazard if not handled and treated appropriately. Distillery spent wash is perceived as one of the most caramelized and recalcitrant waste with organic content and low pH. Previous research paper showed that "The main problem in treating distilleries spent wash is its colour which contains about 2% of the dark brown recalcitrant pigment known as "Melanoidin" **[11]**. The present study reports that "Microbial treatment effluent may be less toxic and safe device for removing Melanoidin from distillery effluent. The bacterium *B.subtilis* has been found effective in decolourising Melanoidin, previous results showed 61.1% decolourization after 8 days incubation period **[14]**. In our research work decolourization at 20% spent wash concentration has shown 44.54% decolourization in 3 days incubation period. Direct disposal of distillery effluent in rivers and on land results into pollution of natural bodies and agricultural land, which consequently lose their fertility **[15]**. The present study shows that after treatment of distillery effluent it can be disposed into rivers or lakes and also used as a liquid biofertilizer when enriched with microbes.

CONCLUSION

The Present study about the physicochemical characteristics reveals that the distillery effluent has a very high load of pollutants. The spent wash was dark brown colored, highly acidic in nature and had very high values of solids, electrical conductivity, hardness, sulphates, chlorides, phosphorus compounds, COD content while DO was found to be nil. All physico-chemical values showed decrease when spent wash was diluted and treated.

The Mixed consortium (*Pseudomonas aeruginosa, Proteus vulgaris, and* Two *Bacillus isolates*) have ability to decolorize and degrade Melanoidin in presence of little amount of carbon and nitrogen sources within a very short incubation period. Therefore, it will be beneficial at industrial level for treatment of distillery effluent at economical level. Overall study reveals that spent wash contains high organic nutrients which are beneficial for the growth of plants and in agricultural sector as fertilizer. It was interesting to note that mixed consortium helps in degradation of toxic pigment and decolorization.

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