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Enhanced Degradation of Melanoidin and Caramel in Biomethanated Distillery Spentwash by Microorganisms Isolated from Mangroves

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Abstract: Four microorganisms isolated from samples of mangrove areas were found to be promising and significantly decolorized spent wash. Incidentally, they showed higher phenol degradation and COD reduction as well. The individual colorants imparting the color to spent wash were fractionated. Degradation of melanoidin and caramel was confirmed by UV and FTIR spectral analysis. Decrease in OD of melanoidin and caramel at their λ max and appearance of new peaks and changes in functional groups of compounds in the IR spectra with respect to control suggest their degradation. K1 was the best melanoidin (77%) as well as caramel (54%) degrader followed by other three isolates (Ku3, Rtb2 and EB4).

Key words: Melanoidin · Caramel · Molasses · Pollutants · Spectral analysis

INTRODUCTION

Current population explosion globally urges enlargement of industrial sectors resulting in pollution of water, air and soil. The discharge of pollutants into the environment from various industries poses a threat to living organisms resulting in a greater environmental stress. One such industry of rapid development is the distillery industry. There are 295 distilleries in India, producing 2.7 billion liters of alcohol and generating 40 billion liters of vinasse (spent wash) annually. Spentwash is the wastewater disposed from the distillery industry.

Dark brown color of distillery vinasse is mainly due to the presence of compounds such as melanoidins, caramel, furfurals *etc.* Melanoidin is known to constitute about 2 per cent of vinasse and is formed by the reaction between amino acid and carbohydrate called Maillard reaction [1]. These highly colored components reduce sunlight penetration in rivers, lakes or lagoons which, in turn, decrease both photosynthetic activity and dissolved oxygen concentration causing harm to aquatic life. Disposal of vinasse on land is equally hazardous causing a reduction in soil pH and inhibition of seed germination. The colored compounds in vinasse have antioxidant properties and become toxic to microorganisms.

Although color can be removed by physical and /or chemical methods, they are expensive and environmentally prohibitive. A number of clean up technologies have been put into practice and novel bioremediation approaches for treatment of distillery spent wash are being worked out. Potential microbial (anaerobic and aerobic) as well as physicochemical processes as feasible remediation technologies to combat environmental pollution are being explored [2].

Biological methods offer an excellent alternate for decolorizaiton and bioremediation of vinasse due to their low cost, environmental friendly and publicly acceptable treatment. A number of biological processes such as bioadsorption and biodegradation have been reported having potential application in color removal from vinasse [3, 4, 5]. A wide variety of aerobic microorganisms capable of decolorizing vinasse include bacteria, fungi, cyanobacteria and yeasts.

Ohmomo *et al*, 1982., [6] screened several fungi, isolated from the tropical soils of Japan, for their ability to decolorize molasses malanoidin and observed *Aspergillus*

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G-26-6 to be the most efficient strain. It resulted in an almost constant decolorization yield of about 70 per cent even in a continuous jar fermentor. In another experiment, *Aspergillus niger* VM2 reduced vinasse color up to 80 per cent [7]. The strain degraded individual colorants like melanoidin, caramels and alkaline degradation products by 21 to 22 per cent.

In the present investigation, we attempted to biodegrade the coloring components in biomethanated spentwash using microorganisms isolated from mangrove plants in South India.

MATERIALS AND METHODS

Isolation of Decolorizing Microorganisms from Mangroves: The decaying plant samples and water samples were collected from the mangroves of Kumta in Karnataka, South India. Bacteria, fungi and actinomycetes were isolated by employing standard serial dilution plating technique [8]. The isolated microorganisms were further purified by subsequent sub-culturing and maintained on slants at 4°C. For initial screening, the isolates were inoculated (2%) to test tubes containing 20 ml of distillery spentwash (20% concentrated). Glucose (1%) was added as the readily available carbon source for the isolates [9]. The test tubes were incubated at 30°C under aerobic conditions and observed daily for any reduction in color of spentwash. The test tubes showing some decolorization were reinoculated successively at weekly intervals to assess their decolorization stability.

Determination of Decolorization by Microorganisms: Samples were drawn at regular intervals for measurement of decolorization. For assaying for decolorization, the samples were centrifuged ('Remi') at 12,000 rpm for 10 minutes. The extent of decolorization in each sample was measured by reading absorbance at 475 nm using a spectrophotometer (Systronics). The decolorization was expressed as percentage of the original absorbance at the start (T_0) [10].

Screening of Isolates for Decolorization of Increased Concentrations of Spentwash: The promising microbial isolates obtained in the initial screening were screened for decolorization of higher concentrations of spentwash by gradually increasing the concentration from 20 per cent up to 70 per cent.

Quantitative Analysis: Standard melanoidin was synthesized in the laboratory by heating 1 M of glucose with 0.5 M of glycine at 90°C and pH5.5 for 6 h [1]. The absorption maxima for both melanoidin and caramel were measured at 330 and 283 nm, respectively [7] in a double beam U-Vis spectrophotometer and a standard curve prepared.

Biomethanated spentwash was diluted to obtain absorbance maxima within 2.0 range at 330 nm and 283 nm. Initial concentrations of melanoidin and caramel in the spentwash were determined with the help of a standard graph. Similarly, the concentration of melanoidin and caramel were determined in the microbially treated spentwash and the extent of degradation of melanoidin and caramel due to microbial treatment was computed as described by Patil and Dhamankar,(1997) [11].

Analysis of Melanoidin and Caramel Degradation by FTIR: The changes occurred in the functional groups of compounds present in the biomethanated spentwash were studied through FTIR (Nicolet IR 200 model, Thermo Electron Corporation, Korea) spectral analysis by comparing treated samples with the untreated samples. Spentwash (30%) was inoculated with the selected microbial isolates and incubated for 7 days at 30°C. After incubation period, 10 ml from each sample was taken separately and centrifuged at 10,000 rpm for 10 min in order to separate microbial debris as pellet. The supernatant was mixed with 20 ml of chloroform in a separating funnel and the chloroform soluble layer collected in a small china dish. The collected samples were kept on a hot water bath till all the chloroform evaporated. The residue remained in the china dish was mixed with potassium bromide (KBr) and the pellet used for FTIR spectrophotometer measurement [12].

RESULTS AND DISCUSSION

Out of 18 microorganisms isolated from mangrove samples, two fungal isolates (K1 and Ku3) and two bacterial isolates (RTB2 and EB4) were selected as they showed more than 50 per cent decolorization even at 70 per cent concentration of spentwash (Table 1) [13]. These four isolates were used for studying degradation of melanoidin and caramel compounds in biomethanated spentwash. Similarly, Shayegan *et al*. [14] isolated *Aspergillus* from the soil in the vicinity of Bidestan distillery Qazvin in Iran and observed that it possessed a very high decolorization efficiency of 81 per cent. Rodriguez *et al*. [15] used *Pleurotus ostreatus* for the biological treatment of vinasse and wastewater of coffee pulp. They found that the species, in submerged cultivation, produced a biomass of 14.8 g per L in vinasse

Table 1: Screening of selected efficient isolates for decolorization of higher concentrations of spentwash

	Per cent decolorization after 7 days						
	Concentration of spentwash						
Code No.	20%	30%	40%	50%	60%	70%	Mean
Adi_1	80.02	65.47	63.09	62.20	45.40	39.63	59.34
Adi_2	76.43	49.22	4.62	43.23	36.23	28.25	46.49
Ku_1	76.33	51.44	49.00	44.20	39.29	37.00	49.54
Ku_2	83.21	71.28	66.36	54.25	41.61	39.66	59.40
Ku_3	91.32	82.29	79.00	68.21	63.21	58.75	73.80
Hl_1	87.33	72.50	55.56	50.38	41.74	40.12	57.94
Hl_2	79.11	60.11	54.35	50.72	42.52	31.19	53.00
K_1	93.17	85.16	80.26	73.58	68.28	65.49	77.66
Bd_2	77.45	65.34	59.54	44.34	38.41	29.35	52.45
RTF_6	85.15	70.59	67.14	62.18	54.23	43.14	63.74
RTF_{10}	79.34	49.23	43.19	40.14	36.22	30.35	46.41
RTB_2	88.60	80.19	78.19	73.31	64.07	53.42	72.96
GB_5	76.17	49.37	44.35	39.81	35.22	28.21	45.52
GF_4	79.21	68.32	60.36	50.52	47.35	41.41	57.86
EB_4	87.64	81.21	80.12	72.15	65.00	51.23	72.89
EF_2	76.45	51.43	43.10	39.35	35.00	28.45	45.63
EF_6	78.32	45.44	40.13	36.32	30.40	30.10	43.45
$UASDF_1$	61.25	51.40	46.52	39.53	35.27	31.27	44.21
Mean	80.92	63.89	58.66	52.47	45.52	39.28	
		T	D	T × D			
	SEm±	0.086	0.049	0.211			
	CD	0.315	0.179	0.772			

Table 2: Melanoidin degradation by the promising decolorizing microorganisms

	Melanoidin concentration (µg/ml)			
Treatments	Before treatment	After treatment	Per cent mean	Per cent melanoidin degraded
T_1 - UIC	82.48	82.43	82.45	Negligible
T_2 – Inoculated with RTB_2	82.48	29.04	55.76	65
T_3 – Inoculated with EB_4	82.48	20.69	51.58	75
T_5 – Inoculated with K_1	82.48	19.09	50.78	77
T_5 – Inoculated with Ku_3	82.48	27.29	54.88	67
Mean	82.48	35.71		
	A	B	A × B	
Sem±	0.037	0.023	0.053	
CD at 1%	0.141	0.087	0.202	

Table 3: Caramel degradation by the promising decolorizing microorganisms

	Caramel concentration (µg/ml)			
Treatments	Before treatment	After treatment	Per cent mean	Per cent caramel degraded
T_1 - UIC	17.98	17.85	17.97	Negligible
T_2 – Inoculated with RTB_2	17.98	9.72	13.85	46
T_3 – Inoculated with EB_4	17.98	8.79	13.38	51
T_5 – Inoculated with K_1	17.98	8.22	13.10	54
T_5 – Inoculated with Ku_3	17.98	8.77	13.37	51
Mean	17.98	10.67		
	A	B	A × B	
Sem±	0.072	0.045	0.102	
CD at 1%	0.274	0.174	0.389	

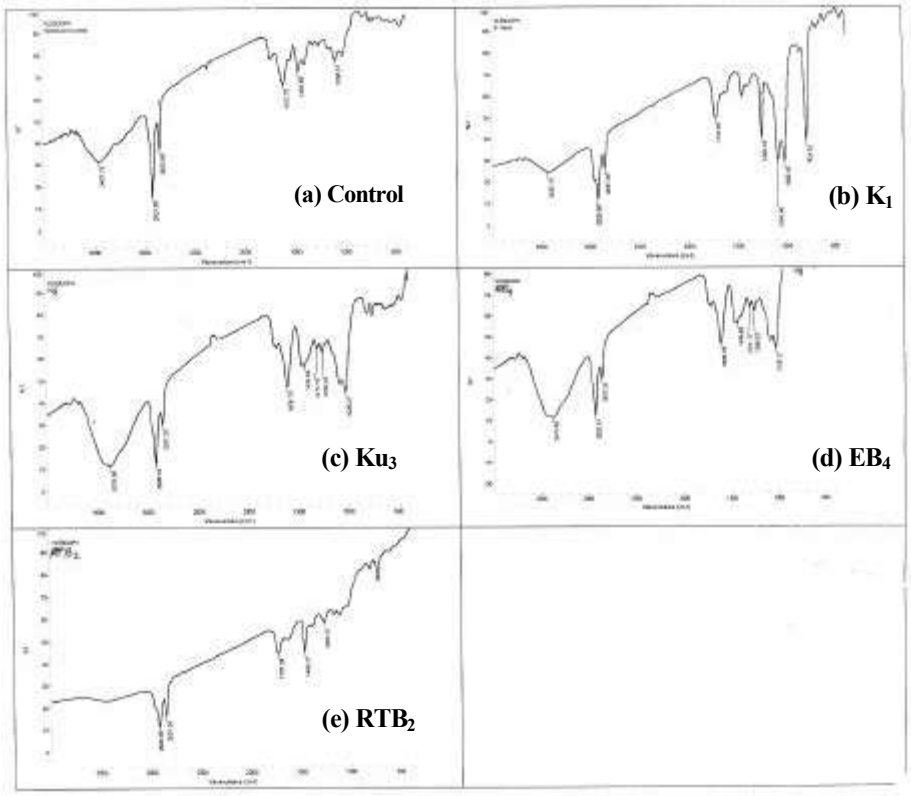

Fig. 1: FTIR spectra of melanoidin and caramel in biomethanted spentwash (a) untreated and (b, c, d and e) treated with different decolorizing isolates

and 5.4 g per L in coffee pulp extract in 10 days of fermentation, finally leading to a significant reduction in COD and the color.

The color imparted to distillery spentwash is mainly due to chemical components like melanoidins, caramels and alkaline degraded products formed by the interactions of reducing sugars and amino acids or degradation of sugars at high temperatures [1]. To develop a strategy for reducing levels of these coloring compounds in spentwash, it is necessary to quantify the concentrations of these specific colorants in the samples. Hence, the quantities of melanoidin and caramel in spentwash (30%) were determined before and after biotreatment by following UV- VIS spectral analysis.

Standard melanoidin and caramel solutions of concentration (10ig/ml), when scanned, showed absorption maxima at 330 nm and 283 nm respectively. This is in support of the absorption maxima reported for these compounds by other investigators [11, 16, 17].

It is fascinating to note that all the four isolates substantially degraded melanoidin and caramel. Isolate K1 degraded the chromophores higher than Ku3, RTB2 and EB4. It degraded melanoidin by 77 per cent (Table 2) and caramel by 54 per cent (Table 3). These results are in agreement with the results of other workers. *Aspergillus niger* UM_2 possessed the ability to degrade both melanoidin, caramel and alkaline degraded products and degraded melanoidin varying from 43 to 51 per cent and caramel from 38 to 41 per cent [7].

Degradation of melanoidin and caramel by our strains was confirmed by UV-VIS and FTIR spectral analysis (Fig. 1). Decrease in optical density of melanoidin and caramel at their λ max (330 nm and 283 nm respectively) and appearance of new peaks in the IR spectra with respect to control suggest their degradation. The observed changes in peaks in IR spectra analysis with respect to control indicate the conversion of complex compounds into simple forms i.e., polymers being broken down to monomers or dimers. An important structural feature of the melanoidin is the presence of conjugated C=C and C=N bonds which impart color to these polymers. The bacterial and fungal strains employed during the present investigation might have brought about the cleavage of ethylinic C=C and azomethine C=N linkages. Therefore, conjugation is broken down due to enzymatic oxidation; hence UV intensity is reduced.

Such cleavage would generate hydroxyl groups (3400cm-[1] band). In conformity to this, intensity of 3400cm-[1] band showed a marked increase in the treated samples compared to the control.

CONCLUSION

Thus, the results of the present investigation have created an understanding about the quantities of individual colorants present in spentwash and their extent of degradation by the efficient microbial strains. These observations could help in developing a technology for the removal of specific colorant from spentwash in future.

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