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Microbial decolorization and bioremediation of melanoidin containing molasses spent wash

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Abstract: *Molasses spent wash from cane-molasses based distilleries contains a brown coloured recalcitrant polymer melanoidin, which if disposed untreated poses a great threat to environment. Microbial decolorization and chemical oxygen demand (COD) reduction was found to be dependent on specific carbon and nitrogen source. Under optimal condition of pH, carbon and nitrogen concentration for each treatment, it was found that Bacillus sp isolated from soil was capable of removing COD (85.35%) and colour (81.10%) from distillery waste to the maximum extent after 9 days at pH 7 in the medium containing 0.5% peptone, 2% glucose and 10% (v/v), followed by Phanerochaete chrysosporium and lowest reduction was obtained by using native microbial consortium.*

Key words*: Molasses spent wash, Bacillus sp, Decolorization. Melanoidin, Chemical oxygen demand*
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Introduction

Molasses spent wash is the waste product produced after the distillation of ethyl alcohol from molasses. This waste is highly hazardous to environment due to its highly acidic nature (pH 4 to 4.3) and high chemical oxygen demand load. According to previous studies, about 10-15 litre molasses spent wash is generated during one-liter ethanol production. Thus, more than 30 billion litre of molasses spent wash is generated annually by 254 cane molasses based distillery in India alone (Kumar *et al.,* 1998).

Melanoidin is a compound, which is present in molasses spent wash and is highly toxic to microorganisms due to its antioxidant property (Fahy *et al*., 1997). This dark brown coloured compound is mainly responsible for high COD value, which is generated during Maillard aminocarbonyl reaction (Wedzicha and Kaputu, 1992). Due to recalcitrant property of melanoidin, its treatment becomes very necessary. It also reduces the fertility of soil by causing manganese deficiency (Chopra *et al*., 2004).

Molasses spent wash also supports the growth of many heterogeneous microorganisms, which can be isolated and ultimately used for the treatment of molasses spent wash. The activities of these organisms are observed under different environmental factors like pH, different carbon source concentration and different nitrogen source concentration. In the present study, *Bacillus* sp isolated from soil and native microbial consortium isolated from MSW were examined at different parameters like pH, carbon, nitrogen sources to optimize the decolorization and COD reduction and results were compared with a standard *i.e.* white rot fungi, *Phanerochaete chrysosporium* (MTCC- 787).

Materials and Methods

The molasses spent wash was procured from anaerobic treatment plant of Doon Valley Distillery Pvt. Ltd., Kuanwala, Dehradun in a plastic can and store at 5°C to minimize further oxidation. It was centrifuged at 8000 g for 10 min to remove suspended solids before using as substrate for further studies.

White rot fungi, *Phanerochaete chrysosporium* (MTCC-787) was collected form Institute of Microbial Technology (IMTECH), Chandigarh and was maintained on basal medium. For isolation of native microflora, 0.1 ml of substrate was spread on the plate having nutrient agar medium and incubated at 37°C for 48 hr. The organisms were isolated and made pure by repeated streaking on different plates. Five different organisms were isolated and the consortium of these isolates has been used as one treatment in this experiment. *Bacillus* species was isolated from soil by serial dilution method and it was characterized as *Bacillus* species after different biochemical tests as per *Bergys Manual of Determinative Bacteriology*.

Experimental set up and decolorization assay:

The influence of various concentrations of carbon, nitrogen sources and pH in presence of *Phanerochaete chrysosporium, Bacillus* sp and native microbial consortium on decolorization and COD reduction of molasses spent wash was examined in triplicate. The basal medium used in these experiments consisted of gl^{-1} glucose, 20, peptone, 5, KH_2PO_4, 1, $MgSO_4$. $7H_2O$, 0.5, Agar, 20 and pH 6 and mixed with 10% molasses spent wash. Different concentrations of glucose (1%, 2%, 3% and 4%) and peptone (0.25%, 0.5%, 0.1% and 1.5%) have been used in the study. A set of controls having no additional carbon or nitrogen source were also

Table - 1: Influence of different carbon concentration as additive in basal medium + 10% MSW on the decolorization and COD reduction by different treatments

Treatment	*Phanerochaete chrysosporium*						*Bacillus* sp						Native microbial consortium					
Parameter	% COD reduction			% Decolorization			% COD reduction			% Decolorization			% COD reduction			% Decolorization		
Glucose (%)	3d	6d	9d	3d	6d	9d	3d	6d	9d	3d	6d	9d	3d	6d	9d	3d	6d	9d
Control*	4.10	10.20	21.07	3.22	10.56	24.07	5.22	9.15	18.70	4.62	16.40	21.16	3.06	11.20	20.40	4.80	14.70	24.28
1	7.20	22.80	43.54	6.21	19.78	50.22	8.12	19.02	33.60	18.03	28.70	47.32	5.02	22.10	39.23	11.96	28.62	52.97
2	10.12	29.80	56.46	15.21	37.97	67.45	16.89	31.94	53.72	13.12	29.57	54.02	6.92	17.23	41.23	10.21	25.90	55.94
3	14.16	29.45	44.98	19.82	39.28	53.77	14.02	24.32	38.70	10.92	19.93	33.55	5.12	21.25	30.99	3.99	22.55	38.55
4	8.12	23.63	34.33	8.02	18.77	42.32	6.07	18.34	23.79	3.98	12.68	27.54	3.17	10.76	20.58	7.02	19.06	27.97

MSW = Molasses spent wash ; COD = Chemical oxygen demand, * = Without C source, peptone @ 0.5%

Table - 2: Influence of different nitrogen concentration as additive in basal medium + 10% MSW on the decolorization and COD reduction by different treatments

Treatment	*Phanerochaete chrysosporium*						*Bacillus* sp						Native microbial consortium					
Parameter	% COD reduction			% Decolorization			% COD reduction			% Decolorization			% COD reduction			% Decolorization		
Peptone %	3d	6d	9d	3d	6d	9d	3d	6d	9d	3d	6d	9d	3d	6d	9d	3d	6d	9d
Control*	2.05	10.15	20.33	6.40	12.70	19.55	4.60	9.25	18.55	5.80	11.92	21.60	3.30	14.56	22.59	4.48	11.73	18.54
0.25	7.12	24.74	45.82	12.02	36.59	46.74	15.01	30.30	50.52	11.81	29.57	40.80	8.10	23.65	38.90	7.19	18.19	33.70
0.50	14.92	38.83	58.40	17.98	40.87	68.23	12.10	20.09	49.74	17.02	35.22	59.86	7.02	18.16	41.07	12.10	27.75	48.77
1.00	12.01	28.72	47.76	19.01	33.48	58.70	5.91	26.68	48.67	9.10	24.42	45.58	10.89	19.9	37.52	4.12	16.45	30.65
1.50	4.10	15.52	30.73	7.02	23.70	35.72	7.02	18.16	34.54	10.98	25.29	37.97	4.01	20.32	28.29	5.99	14.49	23..91

MSW = Molasses spent wash ; COD = Chemical oxygen demand, * = without N source, glucose @ 2%

Table - 3: Influence of different pH in presence of glucose (2%) and peptone (0.5%) on the decolorization and COD reduction by different treatments

Treatment	*Phanerochaete chrysosporium*						*Bacillus* sp						Native microbial consortium					
Parameter	% COD reduction			% Decolorization			% COD reduction			% Decolorization			% COD reduction			% Decolorization		
pH	3d	6d	9d	3d	6d	9d	3d	6d	9d	3d	6d	9d	3d	6d	9d	3d	6d	9d
5	19.8	50.4	67.24	39.20	50.87	69.06	22.12	34.48	54.79	30.80	45.65	60.87	7.18	22.72	36.63	12.10	25.36	44.31
6	41.8	57.08	76.39	38.20	54.78	78.26	18.20	49.16	66.45	40.80	50.36	64.13	12.21	35.76	44.75	8.21	33.70	44.20
7	18.6	42.6	58.10	27.80	43.12	52.12	41.7	64.04	83.35	39.20	64.13	81.10	17.60	35.63	45.59	19.79	40.94	33.99
8	15.2	33.7	49.61	12.80	27.60	39.42	27.19	48.16	64.75	29.78	51.81	64.13	13.21	28.66	38.44	7.92	18.84	28.62

Where as COD = Chemical oxygen demand, d = days

kept for each experiment. Effect of pH on decolorization and COD reduction activity of different microbial treatments *viz.*, *Phanerochaete chrysosporium, Bacillus* sp and native microbial consortium was examined by growing the cultures in basal medium with pH range from 4-7.

In every experiment 5ml of broth culture of *Phanerochaete chrysosporium, Bacillus* sp and native microbial consortium was inoculated to 50 ml of basal medium containing 10% molasses spent wash and incubated at 30°C with shaking. Samples were withdrawn at an interval of 3, 6 and 9 days to determine the per cent decolorization and COD reduction by potassium dichromate method (HMSO, 1986). Samples were centrifuged at 8000 g for 5 min and the supernatant diluted to 5 folds with distilled water. The decolorization activity was measured by determining absorbance of sample at 475 nm wave length and expressed as per cent of the initial absorbance of the sample prior to the treatment.

Results and Discussion

The presence of readily available carbon source is necessary for the growth and ultimate COD reduction and decolorization activity. It is clear from data presented in Table 1 that maximum COD reduction (76.39%) and decolorization (78.26%) activity was achieved at 2% glucose concentration with *Phanerochaete chrysosporium* followed by *Bacillus* sp (83.35 and 81.10%) and minimum was recorded by using native microbial consortium (45.59% and 44.20%) respectively.

It was interesting to note that bioremediation potential of *Phanerochaete chrysosporium, Bacillus* sp and native microbial consortium decreased in media having glucose concentration higher than 2%. Thus, it is clear that the activity of all these three treatments were dependent upon concentration of carbon source. The observation draws support from finding of Aoshima *et al.* (1985), Fahy *et al.* (1997), Kumar *et al.* (1998) Fujit *et al.* (2000), Dong (2005), Singh *et al.* (2005), Saha *et al.* (2005) and Potential and Rodriguez-Malaver (2006).

The decolorization and COD reduction activity of microorganism is also regulated by the nitrogen source present in basal medium. The inoculation of *Phanerochaete chrysosporium* in MSW (10 %v/v) supplemented with peptone (0.5%) which is used as a nitrogen source resulted in about 58.4% and 68.3% reduction in COD and decolorization respectively which was higher than the value achieved by using *Bacillus* sp (50 and 59.86%) and native microbial consortium (47.07% and (48.77%) (Table 2).

In the case of *Bacillus* sp., the percentage COD reduction achieved after 9 days was almost same with 0.25% and 0.50% peptone. A constant increase in reduction and decolorization was observed by the increasing peptone concentrations to 0.5% but thereafter a gradual decline was noticed with increase in concentration of peptone. Similar results were also observed by Chopra *et al.* (2004), Rasmussen and Otsen (2004) and Jain *et al.* (2001).

Thus, it is quite evident from data that percentage decolorization and COD reduction by different treatments in medium supplemented with glucose (2%) and peptone (0.5%) is greatly affected by the change in pH. The maximum percentage of decolorization (81.16%) and COD reduction (83.35%) is observed at pH 7 using *Bacillus* sp, followed by *Phanerochaete chrysosporium* (78.26 and 76.39%) at pH 6 and through native microbial consortium (44.2 and 45.6%) at pH 6 and 7 respectively (Table 3).

Our results indicate that biological treatment can be an effective bioremedial agent that can be exploited to reduce colour and pollution load of distillery effluent.

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