Bioremediation of biomethanated distillery effluent before discharge to reduce contamination of aquatic sources

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Abstract

The effluents from distilleries which contain colored pigments such as Melanoidin, Caramel and Alkaline Degradation Products (ADP) responsible for its dark brown color, high-suspended solids, high concentration of Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD), besides causing aesthetic damage to nearby aquatic sources, are toxic to resident flora and fauna. Anaerobic digestion (Biomethanation) of effluents containing Molasses Spent Wash is one of the treatments followed by distilleries and the resulting dark brown sludge is used as a fertilizer. The effluent after such treatment reduces COD and BOD but is still dark brown in color and is a major problem with distilleries. This paper reports that *Aspergillus oryzae JSA-1,* the natural isolate from soil could decolorize the undiluted biomethanated effluent (BME) effectively by simple adsorption and proved to possess a very high potential in bioremediation of different BME samples. In the study of bioremediation of BME by the column chromatography technique using fungal biomass of *Aspergillus oryzae JSA-1* as a matrix, it was found that the fungal biomass could effectively reduce the total color (99.16 ± 0.09%) of BME samples as well as could reduce most of the important pollution parameters such as COD (90.78 ± 0.22%), sulphates (92.75 ± 0.12%), metals like iron (95.77 ± 0.17%), copper (79.59± 0.08%) and total dissolved solids i.e. TDS (75.59 ± 0.05%) efficiently by biosorption phenomenon.
Keywords: bioremediation, fungal culture, melanoidin, decolorization, biomethanation, distillery effluent, fungal biosorption, chromatography.

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1 Introduction

The effluents from distilleries contain large amounts of molasses spent wash (MSW) which pollutes aquatic ecosystems due to its intense brown color which cuts off light, prevents photosynthesis and causes anaerobic conditions. Next to effluent from paper and pulp mill and tannery, molasses spent wash is a major environmental hazard to land or aquatic sources where they are discharged. Due to the importance attached to prevention of environmental pollution, environmental agencies all over the world are imposing strict regulations for mitigation of pollution from industries. Color removal in such effluents using terrestrial fungi has been reported. Fungi are recognized for their superior aptitudes to produce a large variety of extracellular proteins, organic acids and other metabolites, and for their capacities to adapt to severe environmental constraints [1, 2]. For example, *Aspergillus niger* is the prototypical fungus for the production of citric acid [3 to 5], homologous proteins (especially enzymes) and heterologous proteins [6 to 10]. Moreover, *Phanerochaete chrysosporium* is the model of white-rot fungi for the production of peroxidases [11, 12]. Beyond the production of such relevant metabolites; fungi have been attracting a growing interest for the biotreatment (removal or destruction) of wastewater ingredients such as metals, inorganic nutrients and organic compounds [13–17]. Filamentous fungi show their decolorizing activity in following ways which are due to decomposition by an intracellular enzyme system via production of active oxygen from hydrogen peroxide [18, 19] and/ or the adsorption of coloring components by mycelia [20], especially for the decolorization of melanoidin. The adsorption of melanoidin is the first step of melanoidin decomposition mechanism in microorganisms and in case of *Aspergillus oryzae Y2-32*, due to lack of a melanoidin decolorizing enzyme, the mechanism of decolorization does not continue further [20]. In the present study biomass based decolorization was carried out by successive column chromatography of BME through the biomass of *Aspergillus oryzae JSA-1*.

2 Materials and methods

2.1 Isolation and identification of microbial culture efficient in decolorizing biomethanated distillery effluent

Soil samples were collected from the nearby vicinity of biomethanation plants located in Pune District, in India. These soil samples were screened for growth of micro-organisms showing activity of decolorization of biomethanated distillery effluent. On primary screening twenty fungal strains showed visual decolorization activity on solid medium containing biomethanated distillery effluent. Therefore as the secondary screening, the decolourization activities of these strains in liquid medium with biomethanated distillery effluent, under shaking conditions were examined. Out of these, one strain was found to give maximum decolorization of biomethanated distillery effluent i.e. up to 68%. Identification of the selected strain was carried out by the standard methods with

respect to morphological, cultural, physiological and biochemical characterization [21]. This strain was named *Aspergillus oryzae JSA-1* and was chosen for subsequent experiments of decolorization. The culture was sub cultured and maintained on potato dextrose agar at 4^0C in the refrigerator.

2.2 Collection of different biomethanated effluent samples.

Biomethanated effluent samples were obtained from anaerobic treatment plants set up at four different molasses distilleries in Neera, Pravara, Rahuri and Sanjeevani (Maharashtra state, India). The samples were centrifuged at 10,000 rpm for 30 minutes and refrigerated at 4ºC to avoid further oxidation.

2.3 Preparation of biomass of *Aspergillus oryzae JSA-1*

The medium used for the biomass production contained glycerol 5%, peptone 0.5%, potassium dihydrogen phosphate 0.1%, magnesium sulphate 0.05% and distilled water. The initial pH of the medium was adjusted to 6.The medium was dispensed in 200 ml aliquots in 500 ml conical flasks and sterilized at 121ºC for 20 minutes. The sterile medium was inoculated with the culture (10^7spores/100 ml medium) and incubated at 30ºC on the incubator shaker at 150 rpm for eight days. The mycelial biomass was harvested by vacuum filtration through four layers of cheese cloth, washed extensively with double distilled water and dewatered homogenized biomass was used for further studies.

2.4 Decolorization of biomethanated distillery effluent by mycelial biomass of *Aspergillus oryzae JSA-1* using column chromatography technique

To study the decolorization of BME by bioadsorption mechanism using column chromatography technique, the fungal biomass of *Aspergillus oryzae JSA-1* was prepared, washed extensively with double distilled water and was used to pack the glass columns (column size 10.5 cm x 3 cm). A series of four columns were packed with each of 10 gm wet weight (dry weight 3.404 gm) of mycelia and packed columns were equilibrated with 0.1 M acetate buffer of pH 4.5. 100 ml of undiluted BME (pH 4.5), was loaded on the first column and fraction was collected at a flow rate of 65 ml/ hour. The effluent fraction collected from each column, was further passed through next column serially (Figures 1 and 2). The fraction from last column as well as original BME loaded on the column were then analyzed for the reduction of pollution parameters such as total color, melanoidin, caramel, ADP, COD, TDS, phosphate, sulphate, chlorides, sodium, potassium, calcium, iron and copper by the standard methods for the examination of water and waste water [22, 23], (Table 1). The efficiency of *Aspergillus oryzae JSA-1* to reduce color and COD by column chromatography technique was confirmed by passing different biomethanated effluent samples (100 ml) separately through series of four columns loaded with the biomass in the similar way (Table 2, Figure 3). In order to optimize the process, similar experiments undiluted BME separately through the series of four columns loaded with fungal biomass of *Aspergillus oryzae JSA-1* (Table 3).

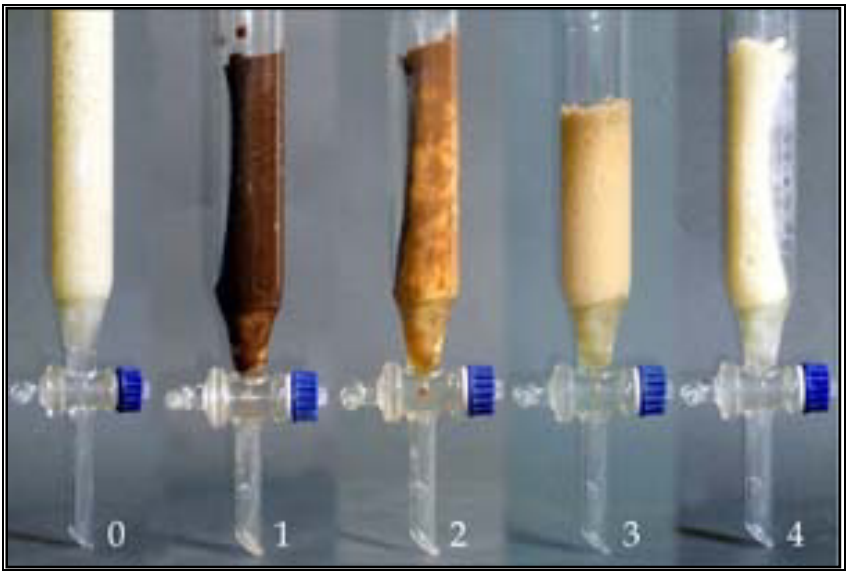

Figure 1: Glass columns packed with wet biomass of *Aspergillus oryzae JSA-1* in column chromatography 0 – before passing the effluent through the packed biomass; 1-4 – after passing the effluent serially from columns 1-4, through the packed biomass.

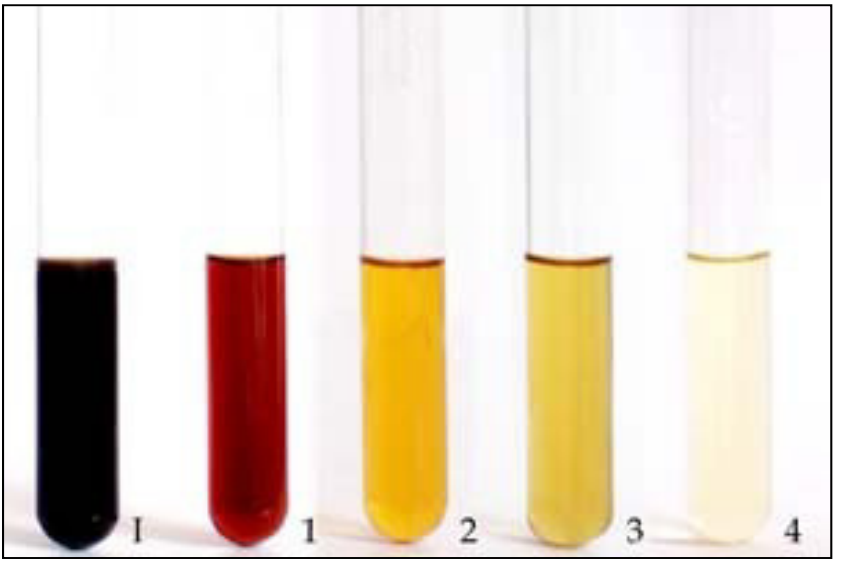

Figure 2: Color of effluent fractions; I – Effluent showing initial color; 1-4 – Effluent fractions showing color after passing through the packed biomass in series of four columns.

Table 1: Chemical analysis and percent reduction of different pollution parameters in biomethanated effluent sample before and after column chromatography.

Parameters	Initial	Final	% Reduction
Color (O.D. 475)	1.19	0.010	99.16 ± 0.09
Melanoidin (w/v %)	0.32	0.010	96.69 ± 0.05
ADP (w/v %)	1.25	0.049	96.12 ± 0.08
Caramel (w/v %)	1.9	0.07	96.30 ± 0.02
COD (ppm)	20,400	1880	90.78 ± 0.22
TDS (ppm)	19, 460	4750	75.59 ± 0.05
Phosphates (ppm)	217.6	74.8	65.62 ± 0.05
Sulphates (ppm)	772.73	56.82	92.75 ± 0.12
Chlorides (ppm)	3678.86	1359.58	63.04 ± 0.13
Sodium (ppm)	345.4	62.8	81.82 ± 0.24
Potassium (ppm)	5030.9	3753.2	25.47 ± 0.06
Calcium (ppm)	320.00	< 8	97.81 ± 0.32
Iron (ppm)	3.67	0.155	95.77 ± 0.17
Copper (ppm)	0.097	0.0198	79.59 ± 0.08

3 Results and discussion

When the study on decolorization of biomethanated distillery effluent was carried out by column chromatography in a series of four columns using mycelium biomass of *Aspergillus oryzae JSA-1* as a matrix, remarkable reduction in total color of the BME sample was seen column wise (Figures 1 and 2).

The results of chemical analysis of the effluent fraction collected from last column of the four columns in series, in the column chromatography are shown in Table 1. Melanoidin, ADP and caramel were found to be reduced by more than 96% and removal of total color was up to 99.16 ± 0.09%. Removal of COD was found to be up to 90.78 ± 0.22%. TDS was reduced to 75.59 ± 0.05%. Levels of all other parameters were also reduced effectively. Phosphates, sulphates and chlorides showed 65.62 ± 0.05%, 92.75 ± 0.12% and 63.04 ± 0.13% reduction respectively. Sodium, potassium and calcium showed around

81.82 ± 0.24%, 25.47 ± 0.06% and 97.81± 0.32% reduction and the trace elements like iron and copper also showed 95.77 ± 0.17% and 79.59± 0.08% reduction respectively.

The efficiency of *Aspergillus oryzae JSA-1* to reduce color and COD by column chromatography technique by passing different biomethanated effluent samples (100 ml) separately through series of four columns loaded with the biomass in the similar way has been shown in Table 2.

The average percent reduction of color was around 72.33% after passing different undiluted effluent samples through first column which was increased to 92.90%, 97.53% and 98.90% in the effluent fractions of second, third and fourth column respectively. While the average percent reduction of COD in the effluent fraction of first column was around 65.73% which was increased to 79.16%, 85.89% and 89.70% in the effluent fractions of second, third and fourth column respectively.

The reduction of color in different effluent samples after passing through the series of four columns is shown in Figure 3.

Table 2: Percent reduction in color and COD after column chromatography of different biomethanated effluent samples.

Undiluted biomethanated distillery effluent sample	Column No.	% Decolorization*	% COD reduction*
Neera	1	75.00 ± 2.34	69.23 ± 1.31
	2	93.87 ± 1.35	82.26 ± 1.24
	3	97.90 ± 1.12	87.51 ± 1.03
	4	99.16 ± 0.09	90.78 ± 0.22
Pravara	1	70.12 ± 1.82	63.12 ± 0.78
	2	93.75 ± 0.79	78.53 ± 1.25
	3	96.68 ± 1.02	86.12 ± 0.98
	4	98.24 ± 0.53	89.17 ± 0.31
Rahuri	1	76.76 ± 2.41	71.22 ± 1.16
	2	93.76 ± 1.11	82.18 ± 0.86
	3	97.97 ± 0.98	87.34 ± 0.23
	4	99.22 ± 0.34	90.11 ± 0.09
Sanjeevani	1	67.43 ± 1.75	59.34 ± 2.11
	2	90.20 ± 1.21	73.67 ± 1.32
	3	97.57 ± 0.63	82.58 ± 1.14
	4	98.96 ± 0.43	88.73 ± 0.46

*With respect to original BME.

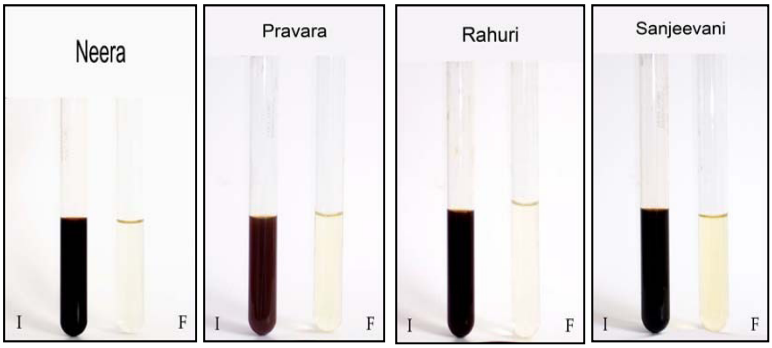

Figure 3: Reduction of color in different effluent samples after passing through series of four columns in column chromatography technique packed with the wet biomass of *Aspergillus oryzae JSA-1*, A – Neera effluent, B – Pravara effluent, C – Rahuri effluent, D – Sanjeevani effluent (I: Initial color; F: Final color).

When the similar experiments were carried out by passing 200 ml and 300 ml of BME separately, to determine the percent reduction of color and COD, the percent reduction of color and COD was found to decrease with increase in amount of effluent loaded (Table 3).

Table 3: Percent reduction in color and COD after passing different volumes of effluent through a series of four columns.

Sr. No.	Volume of undiluted effluent loaded on the column (ml)	% Decolorization	% COD reduction
1	100	99.16 ± 0.09	90.78 ± 0.22
2	200	82.35 ± 1.15	74.26 ± 0.58
3	300	69.87 ± 0.81	63.32 ± 0.63

When the amount of effluent loaded was increased from 100 ml to 200 ml and 300 ml, the average color removal varied from 99.16 ± 0.09% to 82.35 ± 1.15% and 69.87 ± 0.81% respectively and the average COD removal decreased from 90.78 ± 0.22% to 74.26 ± 0.58% and 63.32 ± 0.63% respectively.

4 Conclusion

Among the twenty fungal strains tested, the isolated strain of *Aspergillus oryzae-JSA-1* showed maximum efficiency of decolorization of biomethanated distillery

effluent and it was found to be different in the morphological characterization as compared with the known strains of *Aspergillus oryzae*. Therefore the decolorization experiments on biomethanated distillery effluent were carried out using *Aspergillus oryzae-JSA-1* for development of an efficient process for degradation of coloring compounds in distillery effluent.

From the study of column chromatography using fungal biomass as a matrix, it can be concluded that the fungal biomass of *Aspergillus oryzae JSA-1* could effectively decolorize biomethanated effluent samples by biosorption phenomenon as well as could reduce some important pollution parameters such as COD, sulphates, metals (iron, copper) and TDS efficiently.

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