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ISOLATION OF A STRAIN OF *ASPERGILLUS NIGER*, FROM DECAYING WOOD, CAPABLE OF DECOLORIZING THE DISTILLERY SPENT WASH

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ABSTRACT

In an attempt to search for distillery spent wash (DSW) decolorizing microorganisms, soil samples, deteriorating paints and decaying wood samples were subjected to isolation of bacteria (using nutrient agar), Yeasts (using 3 % malt agar) and Fungi (using Sabouraud's Glucose Agar). In all 30 isolates were obtained. All isolates were screened primarily for their decolorizing ability of distillery spent wash by plate method using modified Sabouraud's Glucose Agar (with various concentrations of spent wash, V/V). The isolate, designated as F2, was selected out during primary screening, was subjected to secondary screening by shake flask culture method. It was found that F2 isolate could grow in and decolorize the distillery spent wash at 1:10 dilution. The fungal culture, F2 isolate, was subjected to morphological and cultural characterization and was identified as a strain of *Aspergillus niger* gr. The identity of the culture was confirmed by referring to the Fungus Identification Service Center.

KEY WORDS : Spentwash, Decolorization, Fungi, *Aspergillus*, Soil, Wood.

INTRODUCTION

Production of ethanol from agricultural materials for use as an alternative fuel has been attracting worldwide interest because of the increasing demand for limited non-renewable energy resources and variability of oil and natural gas prices (Pant *et al.,* 2007). To fulfil this increasing demand the number of distilleries in India has gone very high.There are 319 distilleries in India with a capacity to produce nearly 3.25 billion liters of alcohol (Patel and Jamaluddin, 2018).

Molasses based distillery spent wash is highly acidic, dark brown coloured viscous liquid waste, produced in huge volume(8-15 per liter of ethanol produced) and carries heavy organic and inorganic loading. The dark brown colour is due to the presence of melanoidin pigments which are formed from Maillard reactions of sugar with amino groups of proteins. The waste is very difficult for decolourization and hazardous to environmental health if disposed off untreated (Agrawal and Pandey, 1994). Variety of methods are used for bioremediation of distillery waste water pollution (Kharayat, 2012; Pant and Adholya, 2007)

Since spent wash carry nutrients to support the growth of variety of microorganisms several workers have carried out the work to explore the possibility of using fungi for decolourization and or degradation of distillery spent wash or decolourization of melanoidin pigment of the spent wash (Benito *et al.*, 1997; Ohmomo *et al.*, 1987; Knapp *et al.*, 2001; Raghukumar and Rivonkar, 2001; Gupta & Goel, 2004; Pant *et al.*, 2007; Asgher *et al.*, 2008; Penedo *et al.*, 2009; Ravikumara *et al.*, 2013).

This study was aimed at tapping some sources like decaying wood sample, deteriorated paint sample from walls of building etc. for isolation of fungal strains that are capable of decolorizing of spent wash.

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MATERIALS AND METHODS

1) Collection of the distillery spent wash sample: Distillery spent wash sample was obtained from the primary treatment plant of distillery unit of Sahyadri Co-operative Sugar factory at Yashwantnagar, Tal. Karad, District Satara. A sterile plastic carboy of 5L capacity was used for collection of spent wash. The sample was brought to the laboratory and was stored at 4 ^{o}C till study over.

2) Collection of samples for isolation of Fungi

In total three different soil samples were collected at Distillery unit of Sahyadri Co-operative Sugar factory at Yashwantnagar, Tal. Karad, District Satara, in sterile polythene bags from the nearby area of a lagoon / storage tank, primary treatment plant and from composting plant and were brought to the laboratory. Both deteriorated paint sample from walls of building and decaying wood sample was collected from the campus of Yashwantrao Chavan College of Science, Karad.

Physiochemical characterization of distillery spent wash sample:

The effluent was analyzed for its colour, odour, pH, total suspended solids, chemical oxygen demand (COD) - Dichromate reflux method and biological oxygen demand (BOD)- Winkler's Alkali-Azide method by the using methods as per Standard Methods for examination of Waste and Wastewater (Greenberg *et al.,* (editors) 1998).

Isolation of microorganisms

Each soil sample is first enriched by spentwash containing Nutrient broth and Sabouraud's Glucose broth and then it was serially diluted and was spread inoculated on Sterile Spentwash containing Nutrient agar and Sabouraud's Glucose Agar plates. Plates were incubated at room temperature for 48 hrs. The collected fungal specimens from decaying wood sample were surface sterilized and their sections were taken. These sections were placed separately on sterile 3% malt agar plates. These pates were incubated at room temperature for 96 hrs. The samples collected from the deteriorated paints of plaster of wall from the buildings were subjected to isolation on sterile Sabouraud's Glucose Agar plates and incubated at room temperature for 72 hrs. After incubation period was over isolates were subjected to purification. The purified isolates were partially characterized and preserved at 4^{0}C for further studies. All these isolates were assigned by code numbers.

Screening of the fungal isolates for the decolorization of spentwash

Primary screening of isolated fungal cultures for decolorization of spentwash was carried out by agar plate method, using modified spent wash agar media and incubation the plates at room temperature. The degree of decolorization under colony of isolate was observed from the bottom of the agar plate. Different dilutions of distillery spent wash were used for these studies viz. 1:5, 1:7, 1:10, 1:50 and 1:100 in 100.0 mL.

Secondary screening was carried out at room temperature using Shake Flask Culture method. The selected culture from primary screening was cultivated in 100.0 mL. of spentwash modified Sabouraud's Glucose broth (Glucose – 4%, mycological peptone- 1.0% and pH adjusted to 5.4) prepared in various dilutions of spentwash viz. 1:10, 1:25 and 1:50 in 500 mL capacity Erlenmeyer flask and were incubated in an incubator shaker at 150 rpm. at 37^{0}C for 8 days.

Decolorization assay

Decolorization of spent wash measured as a decrease in optical density measurement at 475 nm on UV Visible spectrophotometer of the treated medium supernatant against uninoculated spentwash medium and expressed as the percentage decrease in absorbance (Gupta and Goyal, 2004).

Identification of promising isolate

The identification of the promising isolate to the species level was done by studying it's morphological and cultural properties and according to standard protocols (Gilman 1957, Domsch et al. 1980, Barnet & Hunter 1986). It was also confirmed by referring to the Fungus Identification Service Center (National Fungal Culture Collection of India (NFCCI) established by DST at Agarkar Research Institute (ARI), Pune.)

RESULTS AND DISCUSSION

Physicochemical characteristics of distillery spent wash

The results of the characterization of the distillery waste(spent wash) are as given in clear from Table 1 that distillery spent wash carries huge organic load

Table 1. Physicochemical characteristics of distillery spent wash

Sr. No.	Parameters	Average Values
1	pH	4.8
2	Odour	Smell of burnt sugar
3	Colour	Dark Brown
4	Total Suspended solids	3397.50 mg/L
5	COD	1,86,000 mg/L
6	BOD, 5 days at 20^{0}C	89,700 mg/L

and is coloured waste.

Isolation of microorganisms from various sources for distillery spent wash decolourizing activity

10 bacterial, 12 mold and 8 yeast strains were isolated from the various samples including decayed wood sample, scrapings from deteriorated paints of walls and soil samples from the nearby area of a lagoon/ storage tank, primary treatment plant and composting plant of distillery unit. All these isolates were designated as A-2, A-3, A-5, B-3, C-1, NII-1, NII-2, NIII-1, NIII-2 and NIII-3 (bacterial isolates),SI-1, SII-1, SII-2, SIII-1, SIII-2, SIII-6, SIII-7 and SIII-9 (Yeast isolates) while SI-2, SIII-4, SIII-5, A-1, A-4, B-1, B-2, D-1,M-1, M-2, F-1 and F-2 (mold isolates).

Primary screening of the isolates for decolorization of spent wash

Among 30 isolates, F-2 isolate has shown good amount of growth and decolorization activity at all dilutions of spent wash (1:5 to 1:100). It is further seen that none of the other isolates showed any decolorization during the incubation period employed, although many of them showed good amount of growth at 1:10, 1:50 and 1:100 dilution of spent wash.

It is interesting to note here that B-2 isolate although did not show decolorization activity has shown good growth at all dilutions of spent wash used. Demonstration of growth in spent wash and decolorization of the spent wash at all dilutions used qualified F-2 isolate as a right candidate for secondary screening. Consequently it was taken up for secondary screening.

Secondary screening of the F-2 isolate for decolorization of spent wash:

Results of the secondary screening of F-2 isolate for decolorization of the spent wash is shown in Fig. 1. It is seen that F-2 isolate showed higher decolorization with increasing dilutions of distillery

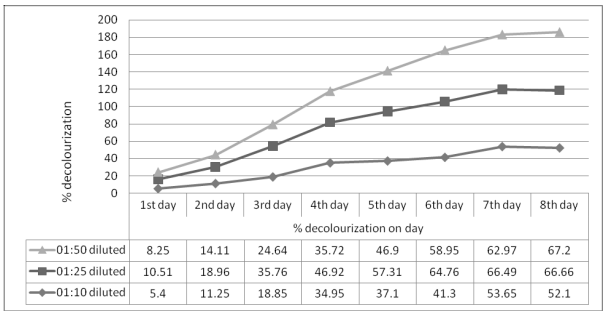

Fig. 1. Percent decolorization of distillery spent wash by F-2 isolate under shake flask culture technique.

spent wash upto eight days of incubation period. This isolate was selected for further investigation by using 1: 10 dilution distillery spent wash.

CONCLUSION

F-2 isolate seems to be promising strain for decolorization of distillery spent wash and has brought about 53.65 % decolorization within 7 days at 37 ^{o}C in 1:10 diluted spent wash concentration. The F-2 isolate is identified as a strain of *Aspergillus niger*. From the above study it is clear that this strain of *Aspergillus niger*, a local isolate obtained from decaying wood to be more efficient in decolorization of spent wash. This strain can be taken up for further studies like media optimization for decolourization and degradation and optimization of other cultural conditions. Also, strain improvement studies can be undertaken. .

REFERENCES

Agrawal, C.S. and Pandey, G.S. 1994. Soil pollution by spentwash discharge: depletion of manganese (II) and impairment of its oxidation. *J. Environ. Biol.* 15: 49-53.

Asgher, M., Bhatti, HN., Ashraf, M. and Legge, RL. 2008. Recent developments in biodegradation of industrial pollutants by white rot fungi and their enzyme system. *Biodegradation.* 19 (6) : 771-795.

Barnett, H. L. and Hunter Barry, B. 1986. Illustrated genera of imperfect fungi,

Benito, G.G., Miranda, M.P. and Santos, D.R. 1997. Decolourization of wastewater from an alcoholic fermentation process with Trametes versicolor. *Biores. Technol.* 61 : 33-37.

Domsch, K. H., Gams W. Anderson, T. H. and Seifert, K. A. 2008. *Compendium of Soil Fungi*, Academic Press, London.

Fitz Gibbon, F., Singh, D., McMullan, G. and Marchant, R. 1998. The eect of phenolics acids and molasses

spent wash concentration on distillery wastewater remediation by fungi. *Process Biochem.* 33 : 799-803.

Greenberg *et al.* (editors) 1998. *Standard Methods for the Examination of Water and Waste Water,* 20th ed., American Public Health Association (APHA), American water works association (AWWA), Water Environment Federation (WEF) : Washington, DC.

Gupta, P. and Goyal, D. 2004. Decolourization of synthetic dyes by *Aspergillus* sp., *Indian J Microbiol.* 44 : 191-195.

Kharayat, Yogita. 2012. Distillery wastewater: bioremediation approaches. *Journal of Integrative Environmental Sciences.* 9 (2) : 69-91.

Knapp, J.S., Vantoch-Wood, E.J. and Zhang, F. 2001. Use of wood-rotting fungi for the decolourisation of dyes and industrial euents. In: Gadd, G.M. (Ed.), *Fungi in Bioremediation.* British Mycological Society. Cambridge University Press, 242

Ohmomo, S., Kaneko, Y., Sirianuntapiboon, S., Somchai, P., Atthasumpunna, P. and Nakamura, I. 1987. Decolourization of molasses wastewater by a thermophilic strain *Aspergillus fumigatus* G-2-6. *Agric. Biol. Chem.* 51 : 3339-3346.

Pant, D. and Adholeya, A. 2007. Biological approaches for treatment of distillery waste water: a review. *Biores. Technol.* 98 : 2321-2334.

Pant, D. and Adholeya, A. 2007. Identification, ligninolytic enzyme activity and decolorization potential of two fungi isolated from a distillery effluent contaminated site. *Water, Air, and Soil Pollution.* 183(1-4): 165-176.

Pant, D. and Adholeya, A. 2007. Enhanced production of ligninolytic enzymes and decolorization of molasses distillery wastewater by fungi under solid state fermentation. *Biodegradation.* 18 (5) : 647-659.

Patel, S. and Jamaluddin, 1918. Treatment of distillery waste water: a review. *International Journal of Theoratical & Applied Sciences.* 10 (1) : 117-139.

Pinedo-Rivilla, C., Aleu, J. and Collado, I.G. 2009. Pollutants biodegradation by fungi. *Current Organic Chemistry.* 13 (12) : 1194-1214.

Raghukumar, C. and Rivonkar, G. 2001. Decolourization of molasses spentwash by the white – rot fungus *Flavodon flavus*, isolated from marine habital. *Applie Microbiology and Biotechnology.* 55(4) : 510-514.

Rajasundari, K., Murugesan, R. 2011. Decolourization of distillery waste water-role of microbes and their potential oxidative enzymes*, J. Appl. Environ. Biol. Sci.* 1 (4) : 54-68.

Ravikumar, R., Vasanthi, N.S. and Saravanan, K. 2013. Biodegradation and decolorization distillery spentwash with product release by a novel Strain *Cladosporium cladosporioides. Optimization and Biokinetics.* 27(3) : 373-383.

Sirianuntapiboon, S., Phothilangka, P. and Ohmomo, S. 2004. Decolourization of molasses wastewater by a strain no. BP103 of acetogenic bacteria. *Biores. Technol.* 9 : 31-39.

Uppal, J. 2004. Water utilization and euent treatment in the Indian alcohol industry – an overview. In: *Liquid Assets, Proceedings of Indo EU workshop on Promoting Ecient Water Use in Agro-based Industries.* TERI Press, New Delhi, India, 13–19.

Watanabe, Y., Sugi, R., Tanaka, Y. and Hayashida, S. 1982. Enzymatic decolourization of melanoidin by *Coriolus* sp. *Agric. Biol. Chem.* 46 (20) : 1623–1630.