IJASRM International Journal of Advanced Scientific Research and Management, Volume 3 Issue 5, May 2018
www.ijasrm.com
ISSN 2455-6378

Physico-chemical characteristics and Microbial decolourisation of spent wash using Indigenous Fungal Isolates

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Abstract

Spent wash is pollution intensive wastewater generated by distilleries. Physico-chemical characteristics such as pH, Electrical Conductivity, Total Solids (TS), Total Dissolved Solids (TDS), Total Suspended Solids (TSS), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD), bicarbonate, carbonate, nitrate and calcium of distillery effluent were analyzed and it was observed that spent wash had high load of chemical and organic pollutants. Soil samples collected from contaminated sites were screened for potent melanoidin degrading fungi. Six fungi were isolated and identified as *Aspergillus flavus*, *A. niger*, *A. terreus*, *Penicillium purpurogenum*, *Penicillium* sp. and Non sporulating hyaline form. The optimum pH, temperature and carbon source for decolourisation of spent wash were standardised. The fungal cultures were inoculated in broth amended with 10% distillery effluent. Maximum decolourisation (72.74%) was seen in Raw Spent Wash (RSW) whereas 65.14% decolourisation was seen in Anaerobically Digested Spent Wash (ADSW). Spectrophotometric analysis of treated effluent confirmed the biodegradation of melanoidin pigments by the fungi.

Keywords: *Spent wash, physico-chemical characteristics, optimisation, fungi, decolourisation, A. flavus*

1. Introduction

Industries are the boon for a country. Economy of the country is maintained by industries. But this rapid industrialisation for sustaining economy lead to the insecure disposal of industrial effluents (Chauhan 2007). Different type of industries such as distillery, sugar industry, paper and pulp, chemical, pharmaceutical and tannery dispose the effluent directly into soil and water bodies, which causes major pollution problem (Sharma, 2013). Most of these industrial effluents are insusceptible to degradation. The major problem faced by the municipalities is the disposal of waste water, especially in large metropolitan areas, where there is limited space availability (Pandey *et al.*, 2008, Chindankumar *et al.*, 2009).

India is the second largest producer of ethanol in Asia and fourth in world. It is also the leading producer of alcohol in the South-East Asian region with the contribution of about 65% of the share. Distillery industry produces alcohol from agro based waste such as sugarcane molasses, sugar beet molasses. But mostly sugarcane molasses is used for the ethanol production. There are about 579 sugar mills present in India (Hukkeri *et al*., 2013). The disposal of the distillery spent wash in to water bodies lead to reduction of penetration of sunlight, caused eutrophication in the rivers or lagoons (Kumar *et al*., 2012). The disposal of spent wash in land continuously made soil acidic, depleted soil manganese availability and inhibited seed germination there by affecting the vegetation of the soil. (Agarwal and Pandey, 1994).

The disposal of wastes from industries is becoming a serious problem throughout the world. Industrial effluents cause pollution due to lack of efficient treatment and improper disposal of effluents. (Santal *et al*., 2011). The major problem in disposing the spent wash is dark brown colour of the effluent. The colour is due a recalcitrant pigment melanoidin. Melanoidin is a natural browning polymer, produced by maillard reaction between reducing sugars and amine compounds which contributes a dark and brown colour to the effluent (Tiwari *et al*., 2012).

Distilleries employ various forms of physical and chemical treatments of waste water. However, these treatments are disadvantageous because of the formation of secondary pollutants, high consumption of chemical agents, fluctuation of colour removal efficiency, high volume of solid waste produced, formation of hazardous by products and intensive energy requirements (Miyata *et al.*, 2000, Moosvi *et al.*, 2005, Pant and Adholeya, 2007 and Tiwari *et al.*, 2012). Microbial treatment is eco-friendly, cost-effective and safe. In recent years, many researchers had tried to use biological processes for removing melanoidin from effluent. Various micro-organisms like yeast, fungi, bacteria and cyanobacterium (Kalavathi *et al.*, 2001, Sirianuntapiboon *et al.*, 2004, Mohana *et al.*, 2009,) were used for the decolourisation of spent wash. In the present research, the use of indigenous fungal cultures for decolourisation of the spent wash was studied.

2. Materials and methods

2.1. Collection of samples

The raw spent wash and anaerobically treated spent wash were collected aseptically from effluent treatment plant of Bannari Amman Sugars, Tamil Nadu, India. The effluent samples were filtered using filter paper (Whatmann No. 1) and stored at 4°C.

2.2. Physico-chemical characteristics of spent wash

To access the degradability of spent wash, different physico-chemical parameters were analysed such as pH, COD, BOD, Total Solids (TS), Total Dissolved Solids (TDS), nitrate and calcium according to the standard methods (APHA, 2012).

2.3. Isolation and screening of fungal cultures

Soil samples were collected from the disposal site of distillery unit for screening microorganisms with melanoidin degrading ability. Fungi were isolated on Potato Dextrose Agar (PDA) medium. Pure cultures were maintained by repeated culturing. The tolerance of fungal cultures towards spent wash were analysed on PDA media amended with 1% spent wash **(Chavan, *et al.*, 2006)**. The potential fungal isolates were identified morphologically.

2.4. Decolourisation assay

A loopful of fungal culture was inoculated in the broth (sucrose - 10g, Potassium dihydrogen phosphate- 1g, Ammonium nitrate - 1.8g, Magnesium sulphate- 0.5g, 10% distillery spent wash, at pH 7) for decolorization of spent wash and was incubated in orbital shaker for 72hrs at 40ºC. The supernatant of the centrifuged sample was read at 475 nm using spectrophotometer. The decolourization was expressed as follows (Pazuoki *et al.*, 2008 and Tiwari *et al.*, 2012).

$$\text{Percentage Decolourization} = \frac{\text{Initial Absorbance} - \text{Final Absorbance}}{\text{Initial absorbance}} \times 100$$

2.5. Optimisation of culture condition for decolourisation

The selected isolates were optimised for various growth parameters required for the decolourisation activity. These parameters include pH (5, 6, 7 and 8), temperature (35ºC, 40ºC, 45 ºC and 50ºC) and carbon source (Dextrose, sucrose and fructose).

3. Results and discussion

3.1. Physico-chemical characteristics of spent wash

The pH of the Raw Spent Wash (RSW) was found to be 5 and that of Anaerobically Digested Spent Wash (ADSW) to be 7. The electrical conductivity was found to be 29.060 mS/cm and 18.777mS/cm in RSW and ADSW respectively. The total dissolved solids in RSW were found to be less as compared to ADSW (Table 1). The total solids in the RSW were found to be 12872mg/L and it was 14727mg/L in ADSW. A very high BOD (78516mg/L) and COD (146125mg/L) were recorded in RSW, and it was 5744mg/L and 24704mg/L in ADSW respectively. Carbonate and bicarbonate were present in ADSW and were absent in RSW. Nitrate and calcium were found to be 39mg/L and 2799mg/L respectively in RSW whereas 16mg/L and 2419mg/L respectively in ADSW. Similar trends in physico-chemical characteristics of spent wash were reported by Kumar and Chandra (2004) and Chaudhary *et al*. (2013). The findings of Ansari *et al*. (2012) revealed high load of chemical and organic pollutants in RSW and ADSW.

Table 1: Physico-chemical characteristics of Raw Spent Wash and Anaerobically Digested Spent Wash

Parameters	Raw spent wash	Anaerobically digested spent wash
pH	5	7
EC (mS/cm)	29.06	18.771
TDS (mg/L)	12704	14670
TSS (mg/L)	168	57
TS (mg/L)	12872	14727
BOD (mg/L)	78516	5744
COD (mg/L)	146125	24704
Nitrate (mg/L)	39	16
Calcium (mg/L)	2799	2419
Bicarbonate (mg/L)	-	13183
Carbonate (mg/L)	-	13381

IJASRM International Journal of Advanced Scientific Research and Management, Volume 3 Issue 5, May 2018
www.ijasrm.com
ISSN 2455-6378

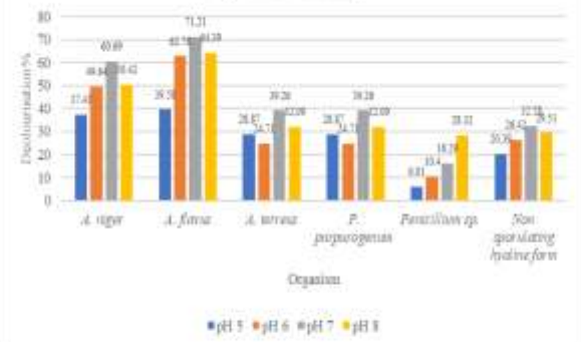
3.2. Isolation, screening and identification of fungal cultures

A total of six fungi were isolated from the soil of distillery disposal site. Secondary screening was done on spent wash amended PDA medium. All the six isolates showed clear zone around the colony. These isolates were considered as effective for decolourisation. The fungal cultures were identified as *A. flavus*, *A. niger*, *A. terreus*, *Penicillium purpurogenum*, *Penicillium* sp. and Non sporulating hyaline form.

3.3. Effect of pH on decolorization of spent wash

Effect of different pH of the medium (5-8) was assessed for spent wash decolourisation by fungi. *A. flavus* (71.21%) showed highest decolorization of spent wash at pH 7. *A. niger* showed 60.69% decolourisation of spent wash at pH 7, whereas least decolorization activity was observed in *Penicillium* species (16.29%). Mirinda *et al*. (1996) reported that *A. niger* showed maximum decolourisation at pH 5. Ravikumar *et al.* (2011) reported that best decolourisation activity was recorded between pH 5-7 by *Cladosporium cladosporioides*. Angayarkanni (2001) reported highest decolourisation between the pH range of 6.5-7 in *A. niveus*. The results of Pazuoki *et al*. (2008) revealed that *Trametes versicolor* had a best decolourisation percentage with an initial pH of 6, and in contrast *Phaenerochaete chrysosporeum* had a higher percentage decolorization with an initial pH of 4. However, significant decolourisation was obtained in the optimal range of 5-7 which confirmed the influence of pH in colour removal of spent wash.

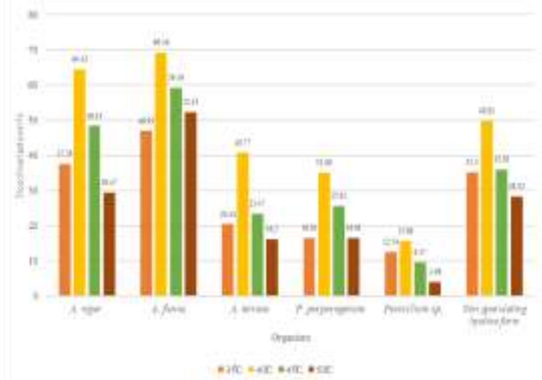
Fig 1: Effect of pH on decolourisation of spent wash by different fungi



3.4. Effect of temperature on decolorization of spent wash

The cultures were active at all the temperatures. The maximum decolourisation was noticed at 40°C. *A. flavus* showed best decolorization at 40°C (69.16%), followed by 45°C (59.19O%) and 50°C (52.33%), thereby showing the best tolerance when compared to other organisms (Fig 2). *Penicillium* sp. showed less tolerance towards various temperatures. Based on the results obtained, the optimum temperature was deduced as 40°C for decolourisation of spent wash. Further, increase in temperature above 50°C, did not affect the decolorization efficiency of the cultures. Some workers have reported the higher biomass attained within 24-48 hours with fast decolourisation at a temperature range of 25-40°C (Ravikumar *et al*., 2012, Jiranuntipon *et al*., 2008).

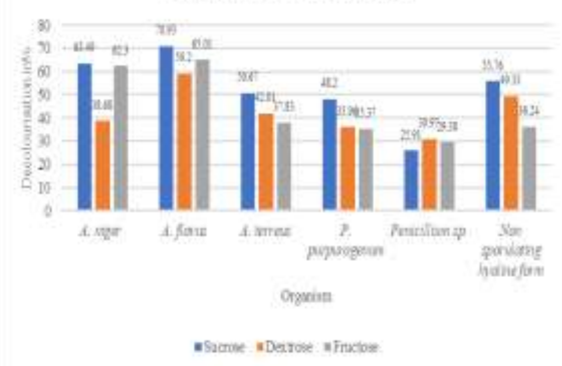
Fig 2: Effect of temperature on decolorization of spent wash by different fungi



3.5. Effect of carbon sources on decolorization of spent wash

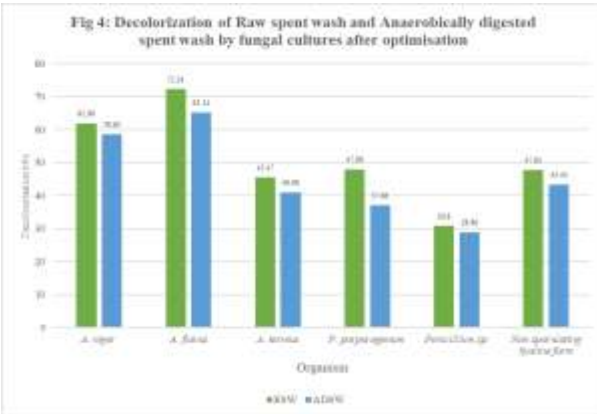
The influence of three carbon sources (sucrose, dextrose, and fructose) on decolorization of spent wash were examined. Sucrose was found to be the best carbon source for spent wash decolourisation. The highest decolourisation was observed at 70.93% by *A. flavus* on sucrose supplementation (Fig 3). *A.niger* exhibited 63.49% decolourisation on sucrose supplementation. The least decolourisation effect was observed in *Penicillium* sp. (25.91%). Angayarkanni *et al*. (2003) observed that sucrose and sugarcane bagasse were best carbon source for *A. niveus*. In contrast the results of Ravikumar *et al*. (2011) revealed fructose was the best carbon source for *C. cladosporioides*.

Fig 3: Effect of carbon source on decolorization of spent wash by different fungi



3.6. Decolorization of Raw spent wash and anaerobically digested spent wash by fungal cultures

In the present study, *A. flavus* was found to be the best isolate for decolourisation of spent wash. It showed highest decolourisation of RSW (72.24%) and (ADSW 65.14%). The least decolourisation activity was observed in *Penicillium* sp. (30.8% in RSW and 28.86% ADSW) (Fig 4). From the experimental results, it was analysed that the decolorization activity by the fungal cultures were high on RSW when compared to ADSW. Shayegan *et al*. (2005) had also reported highest decolourisation (75%) by an *Aspergillus* sp. in aerobically treated distillery wastewater. Mirinda *et al*. (1996) reported 69% colour removal in batch process in *A.niger*.



4. Conclusion

In the present investigation, RSW and ADSW was found to have high organic and inorganic pollutants. *A. flavus* was observed to have high decolourisation activity of RSW and ADSW under optimal conditions. Since the fungi was isolated from the spent wash contaminated soil, they were found to be more effective in the decolourisation process. The potential of these fungal cultures to remove colour in short incubation period is a promising application for the development of cost effective and environment safe technology for distilleries.

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