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OPTIMIZATION OF MEDIUM COMPOSITION FOR ASPERGILLUS NIGER F-2 FOR IT'S DECOLORIZING AND COD REDUCING ACTIVITY OF THE DISTILLERY SPENT WASH

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ABSTRACT

During the secondary screening program of distillery spent wash decolorizing fungi a strain of *Aspergillus niger* F-2 that could decolorize and grow in 1:10 diluted distillery spent wash was obtained. The culture was taken up for further studies for optimization of medium composition for the growth and decolorizing activity of the organism. The Optimization studies include the optimization of pH, inoculum size, carbon and nitrogen source, incubation temperature and incubation period. The optimum pH, inoculum size, temperature and incubation period were found to be 6.00, 2.5%, 35 °C and 3 day respectively. Glucose and peptone were found to be the best carbon and nitrogen sources respectively. Glucose was found to be the best at the concentration of 2.5 % while peptone was found to be the best at 0.1 % concentration. The fungal culture under optimal conditions was found to give maximum decolorization of the 1:10 diluted spent wash to the extent of almost 79%. This isolate also showed the degradation as evidenced from the reduction in the term of COD mg/L to the extent of 58.06% within three days.

KEY WORDS : Decolorization, Optimization medium, Distillery, Spent wash, COD, *Aspergillus niger*

INTRODUCTION

Distilleries producing alcohol are of great concern from environmental pollution point of view, as on an average of the 8-15 L of effluent (spentwash) is generated from them for every liter of alcohol produced. Distillery spent wash is produced as liquid waste in huge amount and carry heavy organic and inorganic load. It is dark brown colored highly acidic and viscous liquid waste (Das and Bhattacharya, 1992). The dark brown colour of this waste is mainly due to the presence of melanoidin pigment. Melanoidins are formed by the maillard amino –carbonyl reaction between reducing sugars and amino acids (Wedzicha and Kaputo, 1992)

Physiochemical methods of removal of Melanoidins have their own limitations.

Disposal of the distillery spentwash into water bodies is hazardous as it has great pollution potential affecting the aquatic life in the water bodies like streams and rivers.

Distillery spentwash also supports the growth of many heterogeneous microorganisms which can be isolated and ultimately used for the treatment of spentwash.

Therefore distillery spentwash could be amenable to microbiological methods of treatment. In fact several workers have reported the role and potential of some specific microorganisms in the degradation and or decolourization of spentwash (Wetanabe *et al.*, 1982, Raghukumar and Rivonkar, 2001; Chavan *et al.*, 2006; Fahy *et al.*, 2007.; Singh *et al.*, 2007; Naik *et al.*, 2009; Singh and Dixit, 2010; Ravikumar *et al.*, 2011; Ravikumar *et al.*, 2013). Some workers have

reported the role and potential of some microorganisms for the decolorization of the predigested or an aerobically digested distillery spentwash (Aoshima 1985; Kumar *et al.,* 1997; Jain *et al.,* 2002). In the present study optimization of culture conditions for the decolourization and COD reduction in distillery spentwash, by a fungal isolate *Aspergillus niger* F_2 culture that was obtained during the secondary screening of programme of distillery spentwash decolourizing fungi was carried out.

MATERIALS AND METHODS

Organisms: A fungal isolate that was obtained during secondary screening programme of the distillery spent wash decolourizing fungi, showing promising results, was initially designated as F_2 isolate and later on identified as strain of *Aspergillus niger* was used for optimization studies.

Optimization of carbon source

Five different carbon sources viz. Glucose, fructose, lactose, molasses, sucrose added to the growth medium were tested for their effects on decolorization of spentwash. All these carbon sources were amended to the spentwash at 1.5, 2.0 and 2.5 percent level (Sirianuntapiboon *et al.*, 2004). The selected efficient culture was inoculated and incubated for eight days.

Optimization of nitrogen source

Different nitrogen sources like peptone, urea, $(NH4)_2SO_4$, $NaNO_3$ and yeast extract were tested for their effects on decolorization of spentwash. All these nitrogen sources were added to spentwash at 0.1, 0.3 and 0.5 % level. The selected efficient culture was inoculated and incubated for eight days. The optimized concentration of carbon source was added to all. The best nitrogen source was arrived at based on decolorization potential.

Optimization of pH of the medium

To determine the optimum pH for efficient decolorization, the pH of the spent wash modified medium was adjusted to 4.0, 5.0, 6.0, 7.0 and 8.0 and inoculated with the selected culture. The decolorization yield obtained at these pH levels was compared with that of the spentwash (pH unadjusted).

Optimization of inoculum size

The selected culture of *Aspergillus niger* (F_2 isolate) was inoculated to spentwash at different levels viz. 1.0, 2.5, 5.0 and 10.0 percent to optimize its level for maximum decolourisation.

Optimization of temperature

To find out the optimum temperature for efficient decolorization, the distillery spentwash modified medium was inoculated with selected culture and incubated at different temperature viz. 25 °C, 30 °C, 35 °C and 40 °C.

RESULTS AND DISCUSSION

The medium composition for the decolorizing activity of distillery spent wash by *Aspergillus niger* F_2 was examined as follows:

Carbon source

The results of the effect of various carbon sources are depicted in the Figure 1. It is clear from figure 1 that supplementation of glucose at 2.5% concentration could lead to the maximum decolourization of spent wash (to the extent of 71.05%) by the fungal isolate – *Aspergillus niger* (F_2 isolate) The observations draw support from findings for *Phanerochaete chrysosporium* (Guimaraes *et al*., 2005) and *Citeromyces sp*. WR-43-6 and showed that the reducing sugar in culture broth rapidly decreased with decreasing color intensity (Sirianuntapiboon *et al*., 2004). Adikane *et al*. (2006) also concluded that the utilization of organic nitrogen and carbon source (reducing sugar) has a critical role in decolorization.

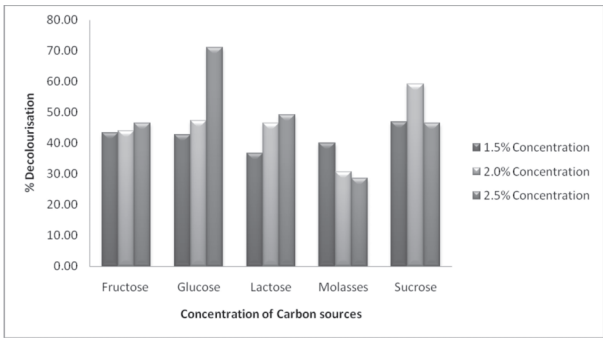

Fig. 1. Effect of different added carbon sources on spentwash decolorization

Nitrogen source

The effect of types and concentrations of nitrogen sources on decolourisation activity of spentwash by *Aspergillus niger* F_2 culture is shown in Figure 2. Out of five nitrogen sources examined, 0.1% peptone

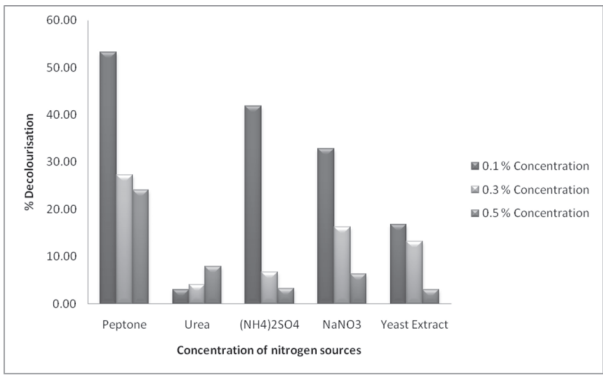

Fig. 2. Effect of different added nitrogen sources on spentwash decolorization

showed significantly best decolorization (53.26%). The next best nitrogen source was 0.1% $(NH_4)_2SO_4$. Urea recorded the least decolorization.

pH

The highest decolorization of the spent wash (61.51%) by *Aspergillus niger* F_2 culture was obtained when pH of spent wash was adjusted to 6.0. However, decolorizing activity decreased significantly when the pH was higher than 6.0 (Figure 3).

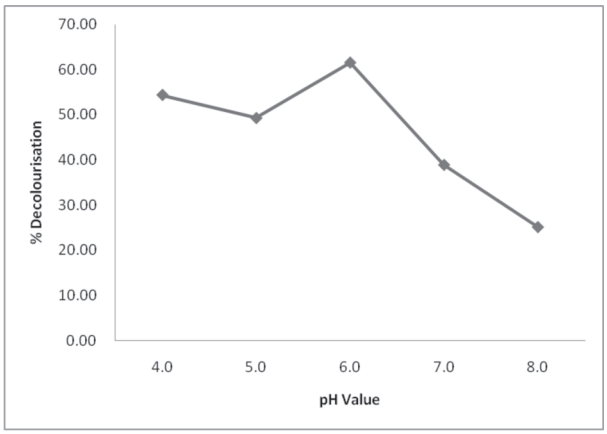

Fig. 3. Effect of pH on spentwash decolorization

Temperature

It is observed that F-2 culture showed highest decolorization activity at temperature 35°C about 54.36 % decolorization (Figure 4).Watanabe *et al.* (1982) also reported maximum decolorization of melanoidin by *Coriolus* sp. at pH 4.5 and at 35 °C.

Inoculum level

Inoculum of different size for *Aspergillus niger* F_2 isolate were tested for their effects on decolorization

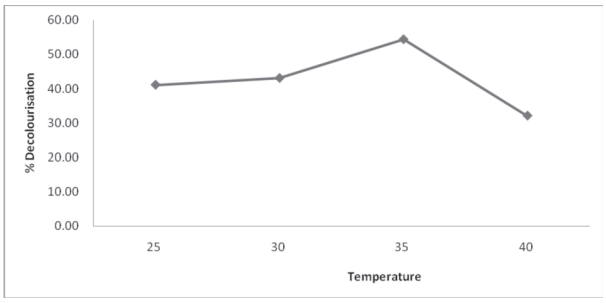

Fig. 4. Effect of temperature on spentwash decolorization

of 1:10 diluted spent wash in presence of optimum levels carbon and nitrogen. The relationship between inoculum size and percent decolorization is shown in Figure 5. It is observed that highest decolorizing activity was obtained with an initial inoculum size of 2.5 % about 52.93. Beyond 2.5%, the decolorization yield did not vary significantly.

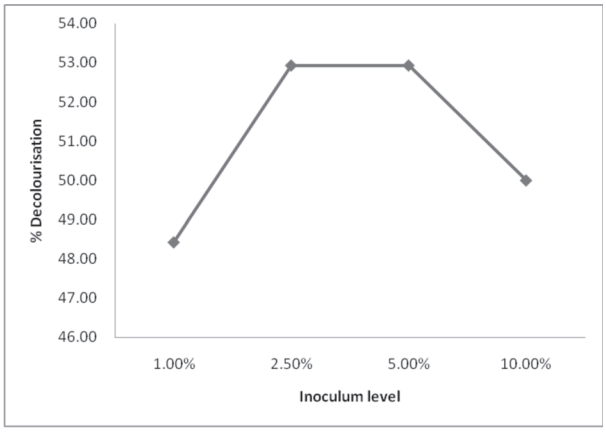

Fig. 5. Effect of inoculum size on spent wash decolourization

From the results of optimisation of various parameters of growth conditions, the medium composed of glucose 2.5%, peptone 0.1%, pH adjusted to 6.0 of distillery spent wash was prepared. It is inoculated with 2.5% inoculum of F-2 isolate and incubated at temperature 35 °C. The results of decolourisation are as shown in Figure 6 and 7. It is observed that *Aspergillus niger* F_2 isolate has brought about 78.84% decolourisation of distillery spent wash within 3 days of incubation period. When both decolorization and COD reduction were monitored as a function of time, the result showed that COD reduction was notable with the increasing decolorization and it was probably upto 72 hrs.

The Chemical Oxygen Demand (COD) of distillery spent wash was determined before and

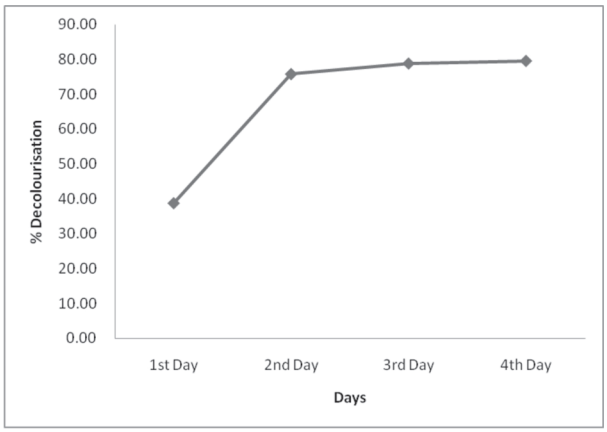

Fig. 6. Percent decolourization of Distillery spent wash (DSW) by using optimized medium and cultural conditions by F-2 isolate.

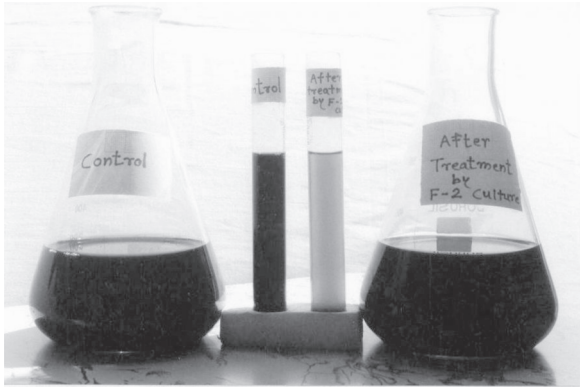

Fig. 7. Decolourization of distillery spent wash by F-2 isolate at optimized cultural conditions.

after treatment with the *Aspergillus niger* F_2 culture. Upon 3 days treatment of spent wash, percent reduction of COD was obtained to the extent of 58.06. The results of COD reduction studies by the F-2 isolate presented in Figure 8.

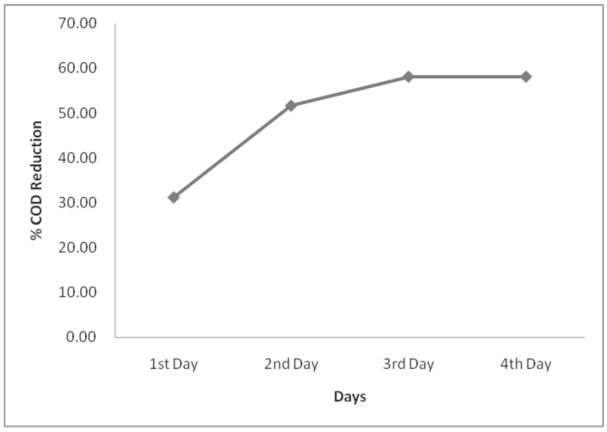

Fig. 8. Percent COD reduction of Distillery spent wash (DSW) by using optimized medium and cultural conditions by F_2 isolate.

Decolourization of spent wash by *Aspergillus niger* F_2 isolate to the extent of almost 79% and COD reduction to the tune of 58% within three days is very significant.

Overall, our results indicate that fungus isolate (*Aspergillus niger* F_2 isolate) could be an effective bioremedial agent that can be exploited to reduce colour and pollution load of molasses based distillery effluent, particularly at places where ample amount of water is available for dilution of spent wash.

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