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Optimization of The Physiochemical Parameters for Enhanced Decolourisation of Congo Red by using Fungal Strains

KEYWORDS: Decolourisation, Congo red, bioremediation, Asperigillus fumigates, Asperigillus Terreus.

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ABSTRACT *In the current study two fungal species are screened from 10 isolates was studied to assess its potential for the decolourisation of an azo dye (Congo red). In this study selected fungal strains, were tested in different culture condition to assess its real potential for bioremediation of dye in effluent. The biodegradation of this dye by these fungus Asperigillus Terreus (KJ522845) and Asperigillus fumigates (KJ522846) shown positive results for the degradation/ decolourisation of the dye. The result shows the optimum parameters for the degradation.*

I. INTRODUCTION

Large amount of wastewater is produced and discharged to the receiving environment during textile process. Strong colour of the effluent is the most serious problem pose the threat to the environment. Azo dyes constitute the largest and versatile class of synthetic dyes used in the textile, industries and represent major components in wastewater. The Ministry of Environment and Forests (MoEF) has prohibited around 42 benzidine based dyes and 70 azo dyes which are capable of releasing any of the harmful amines. (MoEF, Jan 1990) Decolourisation of the effluent is a challenging process to the textile industry, and the potential of microbial decolorizing can be adopted as an effective tool. (Anastasi A1, Parato B, Spina F, Tigini V, Prigione V, Varese G C., 2011) The conventional method for treating the effluent has found to be generating waste product that pose additional serious disposal problem. Microorganisms can breakdown most compounds for their growth and energy need (McMullan. G, Meehan. C., Conneely. A., Kriby N., 2001). This paper examines various fungi, living or dead cells, which are capable of decolorizing the effluents. (Moreira M.T., Mielgo I., Feijoo G.,.Lerna J.M, 2000) Azo dyes are characterized by presence of nitrogen-nitrogen bond (N=N) in its chemical structure. The azo groups are generally connected to benzene and naphthalene rings, but can also be attached to aromatic heterocyclic groups. In order to exploit the potential various process parameters like nutritional sources, pH, and temperature were optimized to develop an economic decolorization process (Meera Gupta, Kumari Manisha, 2012). Degradation analysis was studied using UV–Visible Spectrophotometer, GC-MS and FTIR.

II. MATERIALS & METHODS

A. Wastewater and Chemicals

The effluent was taken from the common effluent treatment plant, kanchipuram, Tamilnadu; this sample was used for isolating indigenous organisms for treatment process. The textile dye Congo red was used throughout the study. All the chemicals were of highest purity and of analytical grade.

B. Microorganisms and Growth conditions

The minimal agar medium is used for isolating the organisms. The medium consists of distilled water, PDA, Yeast extract, Glucose. The medium was then sterilized and autoclaved. It was then incubated for 48 hours.

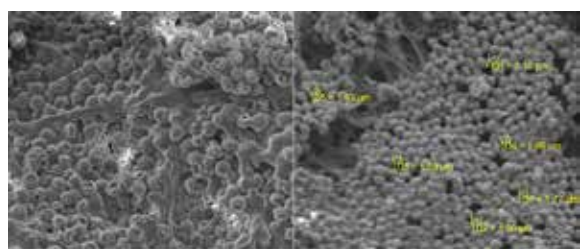

Figure 1 SEM images of fungal strains

MSM were added into the concentrations are Sodium citrate (2.5g/l), KH_2PO_4 (5g/l), NH_4NO_3 (2g/l), $MgSO_4$ (0.2g/l), KCl (0.1g/l). All the isolates were selected for screening of decolourisation of the dye. Inoculum of each isolate was added to 100ml dextrose broth supplemented with dye and incubated at 26°C for 72hrs (Kumar Praveen G.N. and Sumangala K. Bhat, 2012). The effective decolourisation was visually seen those isolates are used for process optimization studies for enhanced decolourisation (Mayur Gahlout, Shilpa Gupte, and Akshaya Gupte, 2013). Two fungal strains used for these study was identified on the basis of morphological characters and sequencing using 16S rDNA sequences, the GenBank accession nos. for Asperigillus Terreus was KJ522845 and for fumigates KJ522846.

C. Optimization of Culture Conditions

The decolourisation process is dependent on the factors such as the dye structure, nutritional sources, pH, temperature, dye concentration, aerobic or anaerobic process and the agitation. (Prachi Kaushik, Anushree Malik, 2011)

III. RESULTS & DISCUSSIONS

Decolourisation of dye by the fungal isolates was optimized with respect to temperature, pH, and for nutritional sources such as carbon, nitrogen sources.

A. Effect of Temperature

100 ml of culture amended with 200 mg/l of dye under different temperature were determined. The rate of colour removal increases with temperature, within a range of 35-37°C. The decline in colour removal activity at higher temperatures is attributed to the loss of cell viability or to the denaturation of the enzyme. (Fig. 2)

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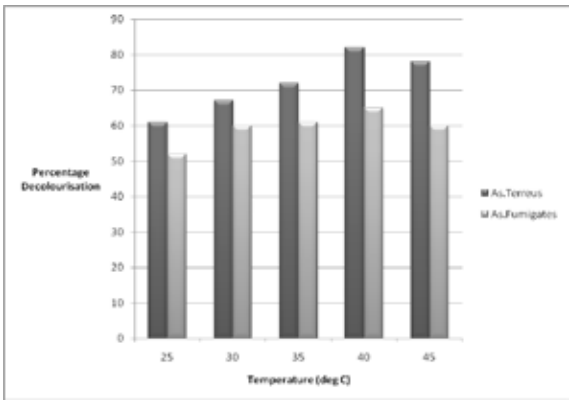

Figure 2 Temperature optimization of decolourisation

B. Effect of pH

100 ml of nutrient broth inoculated with Asperigillus species was amended with 200 mg/l of dye with varying pH from 2–9 were experimented. [6], [7] pH was adjusted using either Hydrochloric acid or Sodium hydroxide. Results clearly indicate that the optimum pH for colour removal is at slightly acidic and the rate of colour removal tends to decrease rapidly at strongly acid or strongly alkaline pH values. (Fig. 3)

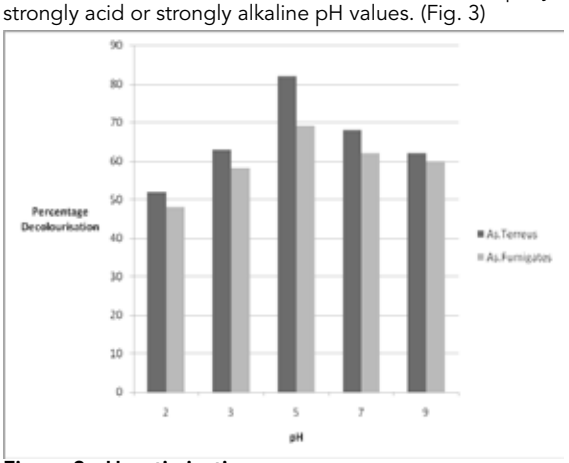

Figure 3 pH optimization

C. Effect of Carbon & Nitrogen Source

The effect of three nitrogen sources Yeast extract, Peptone and beef extract was evaluated. Among the three nitrogen sources, peptone appeared to support the decolorization process by the fungal strains. The effect of carbon sources on decolorization of dye by Asperigillus species was determined that dextrose has as the ideal carbon source for the strains. Fructose recorded least percentage decolorization by all the strains.

D. Chromatography- Mass Spectrum Analysis

GC-MS technique was performed using GC SHIMADZU QP2010 system and gas chromatograph interfaced to a Mass Spectrometer (GC-MS) equipped with Elite-1 fused silica capillary column. An electron ionization energy system with ionization energy of 70eV was used. Helium gas was used as the carrier gas. Mass spectra were taken at 70eV; a scan interval of 0.5 seconds with scan range of 40 – 1000 m/z. Total GC running time was 35 min. The relative percentage of each component was calculated by comparing its average peak area to the total areas. The Metabolites are found to be Ergoline 8 Carboxylic acid, methyl ester, Dasycarpidan- 1-methanol, Ascorbic acid, 2,6-dihexadecanoate.

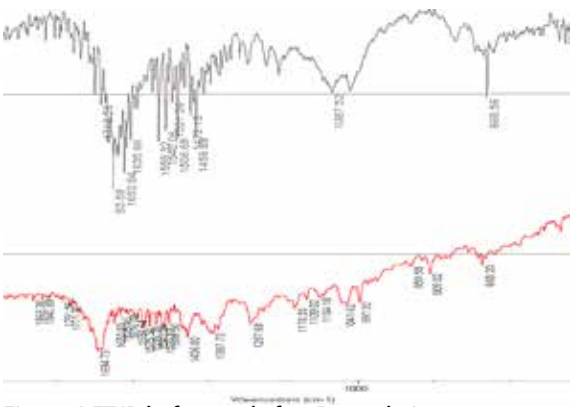

Figure 4 FT-IR before and after Degradation

IV. CONCLUSIONS

These isolate have the effective potential in decolourisation of Congo red, among the fungal isolates the As. Terreus shows the highest percentage of decolourisation of 85% in 72hrs. The strain was capable of decolorizing the azo dye over a pH range of 4–6. The optimum temperature was found to be 35–37 °C. Various other process parameters like additional carbon and nitrogen source were also optimized. UV–Visible analysis has been used to confirm the decolorization of dye and GC-MS and FT-IR confirmed that the degradation has takes place.

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