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Biodegradation efficiency of *Aspergillus awamori* (MTCC-548) against Rose Bengal: A toxic dye for human corneal epithelium.

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ABSTRACT

Biodegradation experiment was performed by growing *Aspergillus awamori* (MTCC-548), a filamentous fungal strain on czapek broth media containing a known conc. of Rose Bengal dye (100 mg/L) at 30±2 °C for a period of 10 days. It was found that incubation of 10 days (3 Days Shaking: 7 Days Static) significantly enhanced the biodegradation efficiency. Results obtained on 10th day revealed that increased biomass is quite helpful in removal of dye from water source by adsorption process. Maximum removal (99.74%) of dye was found on 10th day. The results from the present work clearly demonstrates the potential of filamentous fungal strain towards removal of colored products from waste water.

Keywords: Adsorption, *Aspergillus awamori*, biodegradation, industrial effluents, Rose Bengal.

INTRODUCTION

Dyes are in demand due to their multifunction capabilities in textile and industrial processes. Textile industries are major sources of effluents and eventually results in high waste water generation (Ghoreishi and Haghigh, 2003). The textile industries utilize large volumes of water in their processing operations and generate substantial quantities of waste water (Hutton, 1972). Industries discharge their waste produced during the manufacturing and fabric coloration process into the surrounding rivers and lakes. The textile industry releases about 10 to 15% of the dye, which finds its way into waste water (Rodriguez *et al.,* 1999). Waste water mainly comprised of residual dyes, auxiliary chemicals, surfactants, chlorinated compounds and salts (Pandey *et al.* 2007). Huge amount of synthetic dyes are produced for commercial purpose annually worldwide (Zollinger, 1991). Dyes are used in daily routine work for coloration of fabric products. Out of total dye used for this purpose, approximately 20% part becomes waste due to solubility in water and their inability to bind with the fibers used (Husain, 2010). Dissolved dye in water represents a serious ecological hazard for organisms living in aquatic ecosystem (Maas and Chaudhari, 2005; Robinson *et al.,* 2001).

A number of physical and chemical methods including flocculation, photolysis, chemical oxidation and reduction are extensively used for the waste water treatment (Ansari and Thakur, 2006; Zhang *et al.,* 2002). All these methods have some limitations like: cost, time consuming and requirement of more space for the treatment processes, etc. Biological processes attract the attention of various industries and researchers, due to their significant efficiency to utilize biological activity of microorganisms to degrade toxic chemicals persisting in the environment (King *et al.,* 1998). Potential of microorganisms to decolorize and degrade specific dyes can be adopted as an effective tool and they are considered to be sustainable and eco-friendly (Bhatti *et al.,* 2008). Previous studies showed that fungi are suitable candidates for the degradation of dyes as compared to bacteria. Important fungal biosorbents include *Aspergillus* (Fu and Viraraghavan, 2002), *Penicillium* (Iscen *et al.,* 2007) and *Rhizopus* (Kumari and Abraham, 2007). Keeping in view importance of fungi for degradation and decoloration of dyes the present study has been carried out. In this study, biodegradation efficiency of *Aspergillus awamori* (MTCC-548) against Rose Bengal dye has been studied.

MATERIALS AND METHODS

The materials and methods with respect to chemicals and microbes used and composition of culture media has been given.

Chemicals

Rose Bengal Dye and all other chemicals used in experimentation such as Sodium nitrate ($NaNO_3$), Potassium Chloride (KCl), Magnesium Sulphate ($MgSO_4.7H_2O$), Ferrous Sulphate ($FeSO_4.7H_2O$), Di-potassium hydrogen orthophosphate (K_2HPO_4), Zinc Sulphate ($ZnSO_4.7H_2O$), Copper Sulphate (II) ($CuSO_4.5H_2O$) and Sucrose were purchased from HiMedia.. All reagents used were of analytical grade. Before using, glassware was washed with tap water and sterilized in an oven at 180 °C for 3 h.

Microorganism

Aspergillus awamori (MTCC-548) which is generally recognized as safe (GRAS) was purchased from Microbial Type Culture Collection (MTCC), Chandigarh. For maintenance and further growth of *Aspergillus awamori* Czapek agar medium was used (**Fig. 1**). Spore suspension was prepared by washing the mycelium grown on Czapek agar for 5-6 days at 30±2 °C, with an aqueous solution of

Fig. 1. *Aspergillus awamori* (MTCC-548) grown on Czapek agar medium.

0.1 % (w/v) Tween 80. Spore suspension containing approximately 1×10^5 spores/ml, was used for inoculating the autoclaved media containing dye.

Culture media composition

Four stock solutions were prepared for media preparation. Stock solutions were: **Stock-A** ($NaNO_3$ 40 g/L, KCl 10 g/L, $MgSO_4.7H_2O$ 10 g/L, $FeSO_4.7H_2O$ 0.2 g/L, $FeSO_4.7H_2O$ 0.2 g/L); **Stock-B** (K_2HPO_4 20 g/L); **Stock-C** ($ZnSO_4.7H_2O$ 1g/100ml); **Stock-D** ($CuSO_4.5H_2O$ 0.5g/100 ml). Added equal volume of **Stock-A** and **Stock-B** (50 ml), 1 ml of **Stock-C** and **Stock-D**, Sucrose was used as a carbon source (30g) in flask and prepared final volume 1 L by adding distilled water. In addition to above minerals, Rose Bengal dye was dissolved in media (100mg/L). Culture media was sterilized in an autoclave at 121 °C for 20-25 minutes. Controlled media was prepared in two parts: Czapek broth and Czapek broth + Rose Bengal. The remaining part of media containing dye was divided into ten parts for determination of biodegradation efficacy of *Aspergillus awamori* (MTCC-548) for period of 10 days at 30±2 °C temperature.

Degradation efficiency of *Aspergillus awamori*

The inoculated media containing dye was taken out from incubating conditions after every 24 hours and filtered using Whatmann no. 1 filter paper. The liquid portion was assessed for (%) degradation using Elico UV-Vis Spectrophotometer SL-159 at 550 nm. Degradation efficiency of *Aspergillus awamori* (MTCC-548) against Rose Bengal dye was measured using following equation.

$$\text{Degradation efficiency (\%)} = \frac{\text{Initial Absorbance - Final Absorbance}}{\text{Initial Absorbance}} \times 100$$

RESULTS

When degradation efficiency of the *Aspergillus awamori* (MTCC-548) against Rose Bengal dye (100mg/L) was observed, the maximum degradation (99.74%) was noticed on 10th day of incubation under shaking as well as static condition (3:7 Days, respectively) **(Fig. 2)**. Till date colored compounds in the effluent has been reported less than 20 mg/L. In addition, the production of fungal biomass was found maximum during transfer from 3 days shaking conditions to 7 days static conditions. The combined shaking and static conditions strongly accelerated the degradation efficiency. The increased biomass and maximum surface area provided by fungal

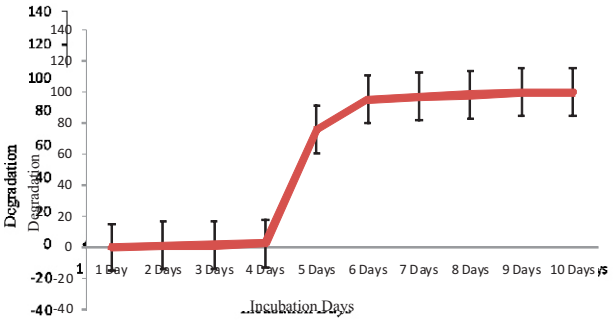

Fig. 2. Effect on incubation time (Days) on biodegradation efficiency of *Aspergillus awamori* (MTCC-548).

Fig. 3. Fungal mat showing adsorption of Rose Bengal Dye.

mat **(Fig. 3)** helps in adsorption of dye on its surface and resulted in clearance of dye containing solution **(Figs. 4a and 4b)**. Present fungal biomass proved to be efficient for dye decolorization and waste water treatment process (Mansur *et al.* 2003).

Fig. 4 (a). Initial Colour of Rose Bengal containing media.

Fig. 4 (b). Clear solution after incubation with *Aspergillus awamori* (MTCC-548) on 10th Day

DISCUSSION

Although decolorization and degradation process is a challenging task for waste water treatment, the results of this study suggest a great efficiency of *Aspergillus awamori* (MTCC-548) in this regard which can be used to remove color from waste waters contaminated with rose Bengal dye. The Rose Bengal dye is degradable under 30±20 °C with a combined shaking and static condition (3:7 Days). Findings from the present study proves the biological utility of process for removing the color from waste water and it can be an alternative and safe method in this type of treatment rather than expensive physical and chemical methods. The results achieved are in conformity with earlier such findings of Mansur *et al.* (2003) and Rodriguez *et al.* (1999) wherein lignolytic fungi have been successfully used for decolorization of industrial dye by making use of their laccase producing capability.

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