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Decolourisation of blue BB by *arcyria cinerea* isolated from the gut of spider (*Achaearanea tepidariorum)*

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ABSTRACT

Synthetic dyes constitute the largest group of dyes used in the textile industry and possess recalcitrant chemical groups such as those of azo and sulphonic acid. Some microorganisms are able to degrade these aromatic compounds. In the present work, decolourisation of culture media containing Blue BB dye by the fungus *Arcyria cinerea* isolated from the gut of spider (*Achaearanea tepidariorum)* was achieved under optimum conditions. The aim of this study was to pre-adapt the microorganism to decolourize the dye under optimum conditions. The optimization was carried out for various parameters like temperature, pH, aeration/agitation, equilibrium time and dye concentrations. The results showed that the optimal decolourization was observed in temperature 25°C, pH 5, agitation 150 rpm, equilibrium time 78 hours and dye concentrations up to 500 mg/L.

KEYWORDS

Spider;
Arcyria cinerea;
Blue BB;
Decolourisation.

1. INTRODUCTION

Synthetic dyes are increasingly used in the textile and dyeing industries because of their ease and cost effectiveness in relation to synthesis, firmness, high stability in relation to light, temperature, detergent and microbial attack. Synthetic dyes are extensively used in a number of industries such as textiles, paper printing, colour photography and food industry[10]. Inefficiency of the dyeing process, poor handling of spent effluent and insufficient treatment of wastes of the dyestuff industries lead to dye contamination of the environment such as soil and natural water bodies[11]. A number of physico-chemical methods such as adsorption, coagulation, precipitation, filtration and oxidation, have been used to treat dyestuff effluents, but these methods have many disadvantages and limitations[4]. It is therefore, important to develop efficient and cost-effective methods for the decolorization and degradation of dyes in industrial effluents and contaminated soil.

Around 10^6 tonnes are produced annually world wide and used extensively in textile dyeing/finishing and also in food, paper and cosmetic industries. The discharge of these highly coloured wastewaters into the ecosystem involves environmental problems. There are more than 10,000 of chemically different synthetic dyes and pigments. Among them, azo dyes are a major class of synthetic, coloured organic compounds and account

for about half of the textile dyestuffs used today[28].

The synthesis of azo dyes is very well established and each year new azo dyes are being developed[23]. Colored effluents from dyestuff and textile industries, the major producers and users of azo dyes, not only produce visual pollution but also cause detrimental effect to life, as they are usually resistant to biological treatment. Azo dyes constitute the largest group of colorants used in industry; however, the environmental fate of these pollutants is not well understood. Azo dyes do not occur in nature and are produced only through chemical synthesis[8,27].

Currently, textile effluents are mainly treated by physico-chemical methods, which are often quite expensive. In addition, therefore causing an accumulation of the dye as sludge creating a disposal problem. Current available technologies have been reviewed[16]. Special attention is given to biological processes because they are cost effective and environmentally friendly. Bant Nigam[2] found that various bacteria and fungi were effective in the decolourization. They have also reported that in many cases the adsorption of dyes to the microbial cell surface as the primary mechanism in the decolourization of effluents.

The microbial degradation and decolorization of dyes have received considerable attention from treating industrial wastewater containing dyes. Azo dyes are the largest class of dyes. Microorganisms that are able to degrade azo dyes have been isolated. Many microbial strains have been isolated to degrade this kind of aromatic compound[7,9,13,15,20]. Most of the metabolic strains have been limited to bacterial genera; however since azo dyes are considerably recalcitrant[19,12] several fungi are able to degrade xenobiotics by cometabolic reactions have been studied[5,7,17].

To protect the environment, strategies that extend the range of xenobiotic compounds degraded in wastewater treatment or the capacities of degradation of microorganisms are required. Bearing this in mind, the dyes used in the present work provide new approaches to enhance the decolourization by *Arcyria cinerea*. Any application of fungal decolorisation of dye waste will require optimization of the operating parameters. The aim of this paper was to study the effect of substrate, nutrient conditions and physiological condition of the fungus *Arcyria cinerea* on the decolorisation of the dyes.

2. MATERIALS AND METHODS

2.1. Isolation of fungi from gut of spider

Potent strains of fungi were isolated from the gut of spider (*Achaearanea tepidariorum).* The spider was washed with 70% ethanol and several times with sterile distilled water to eliminate surface bacteria. All dissections were performed under sterile conditions. After disrupting the walls, the contents of the stomach were collected in sterile eppendorf tubes, containing phosphate-buffered saline which were serially diluted, spread into the surface of sabouraud dextrose agar plates and incubated for 5 days at 28°C in order to record total colony forming units (CFU/ml).

2.2. Fungi and growth

The fungi isolated from the gut of spider was maintained at 4°C on malt agar slants and subcultured every 2 to 4 weeks. The growth medium used contained (g/l) 10 glucose and 10 malt extract. The medium was autoclaved at 15 psi for 30 minutes and cooled at room temperature before use.

2.3. Agar plate screening

Sabouraud Dextrose agar medium was prepared and Blue BB dye was added to a final concentration of 50 mg/L. The plates were inoculated with the cultures isolated and incubated at 30°C for 5 days. Control plates were maintained simultaneously without the culture. The experiments were performed in triplicates for each culture.

2.4. Dyes used for experiments

Blue BB[N-(4-Amino-2, 5-diethoxyphenyl) benzamide] an industrially important dye of commercial grade which is being used in textile industries at Tirupur, Tamil Nadu, India was taken for the experiment. It is a commercial dye and was used without further purification.

2.5. Decolorization experiments with whole cultures of *Arcyria cinerea*

Preliminary experiments for dye decolorisation were carried out in 100 ml Erlenmeyer flasks. In each flask, a dye (Blue BB) concentration of 50 mg/L was taken as standard concentration with temperature 28°C, pH 5.0 at 150 rpm. A fungal mat of 4 mm diameter was

added to each flask. For each experiment, a series of similar flasks were set. A control flask with all the components except the fungal mat was maintained in parallel to obtain abiotic decolorisation, if any. All the experiments as well as controls were run in triplicate. Four milliliter of mixed liquor was drawn at 24 h interval and was centrifuged at 10,000 rpm for 10 min to separate out the fungal biomass. Dye (Blue BB) clearance from the culture fluid (supernatant) was monitored by assaying at A_{600}.

2.6. Decolorization experiments under various culture conditions

The culture conditions were optimized with the basic conditions like temperature, pH, aeration/agitation, equilibrium time, dye concentrations and age of fungus as mentioned earlier. Various conditions such as temperature (20^0C to 40^0C), pH (3.0 to 8.0), shaking (100 to 250 rpm), static (incubation), Equilibrium time (every 6 hrs till 96 hrs), dye concentrations (10, 25, 50,100,200, 300, 400 and 500 mg/L) and age of fungus (1^{st} to 5^{th} day) were studied.

2.7. Analysis

The concentrations of the dye Blue BB was measured at a wavelength corresponding to the maximum absorbance, λ_{max} (600 nm) by means of a UV-Vis spectrophotometer (UV 3210, *Hitachi*, Japan). A standard curve was obtained for dye concentrations ranging from 10 to 150 ppm. The decolorisation was monitored by the % reduction in comparison with controls. Dye solution incubated without the inoculum was taken as positive control and uninoculated culture without dyes was used as negative control (blank) for the dye and the rate of decolorization was calculated.

The efficiency of colour removal was expressed as the percentage ratio of the decolorized dye concentration to that of initial one.

Decolorization (%) = $[\{D_i - D_f\}/D_i] \times 100$

Where Dye (i) = initial dye concentration (mgl^{-1}), Dye (r) = residual dye concentration (mgl^{-1}).

3. RESULTS

3.1. Identification of the isolates

Fungal populations were isolated from the gut of spider using pour plate technique. The identification was done based on morphological and microscopic characters and the fungal isolates were identified as *Aspergillus niger, Penicillium notatum, Rhizopus* sp, *Arcyria cinerea* and *Trametes hirsuta*.

3.2. Primary screening

Initial evaluation of dye decolourization was done using sabouraud dextrose agar medium with a dye concentration of 50 mg/L. By third day, the decolourization of the dye was observed by the formation of clear zones around the colonies. The fungus *Arcyria cinerea* had the highest decolorisation zone to degrade Blue BB. Further screening for the utilization of dye by *Arcyria cinerea* was tested by growing in sabouraud dextrose liquid medium supplemented with the dye Blue BB (50 mg/L) to confirm the potent strain capable of decolorisation. The fungus *Arcyria cinerea* showed maximum decolorization of 72.6%.

3.3. Decolorization experiments under various culture conditions

3.3.1. Effect of temperature and pH

The temperature and pH had a significant effect on decolorization rate for the fungus *Arcyria cinerea* which showed the optimum temperature for decolorization of Blue BB was at a temperature 25^0C at pH 5 (Figures 1 and 2). Above these conditions of temperature and pH

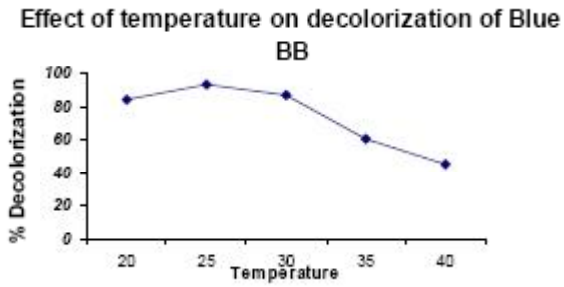

Figure 1

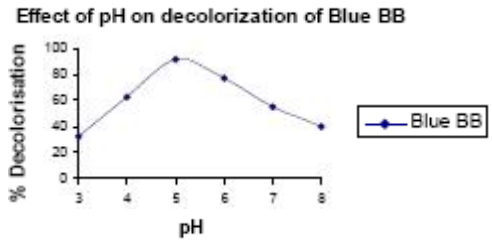

Figure 2

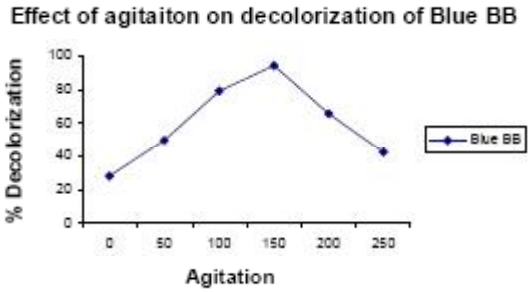

Figure 3

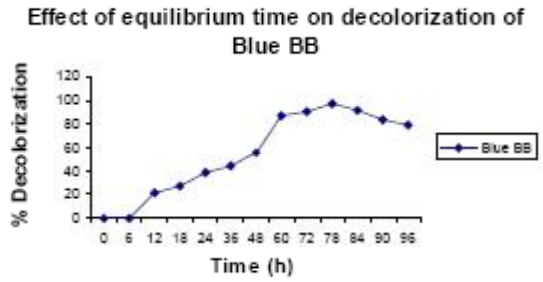

Figure 4

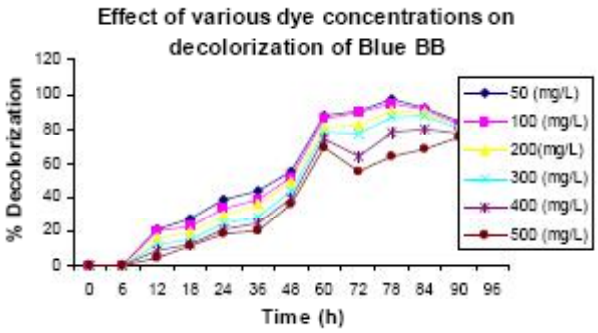

Figure 5

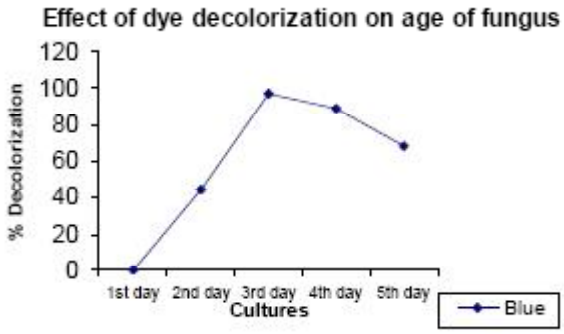

Figure 6

there was decline in decolorization. Fungi have the ability to grow at low pH, normally ranging from 4 to 5. This shows that temperature and pH has a role in the growth of mycelium, adsorption capacity and intensity. This may be due to the reduction of mycelial growth at higher temperatures. The results of present study has been supported by Raghukumar[14] as the effect of pH on colour removal by three marine fungi and effective pH was 4.5. Belsare and Prasad[3] studied the optimum pH of *S.commune* and the most effective pH was 4-5.

3.3.2. Effect of aeration/agitation and static conditions

The influence of dye degradation with different conditions such as shaking and static conditions was demonstrated. The filamentous mats were observed on the surface of the growth medium on third day at static conditions. However, when inoculated flasks were incubated in different shaking conditions uniform mycelial pellets were formed. Agitation at different rpm showed a greater decolorization than static cultures (Figure 3). The highest decolorization of Blue BB was at 150 rpm. This may be due to the oxygen transfer between the cell and medium due to mixing. The formation of the mycelial mat on the surface of the cultures in static conditions restricts oxygen transfer between the cells[21]. The results indicated that the activities of enzymes from the *Arcyria cinerea* were high under shaking conditions.

3.3.3. Effect of equilibrium time

Decolorization of Blue BB was observed maximum at 78 h with 96.82 % removal (Figure 4). The decolorization process involved initial absorption followed by microbial metabolism[24]. Zheng[25] reported the same observation that poly R-478 was initially adsorbed on to the *Penicillium* mycelia and as the culture aged it was degraded from the mycelia.

3.3.4. Effect of initial dye concentration

The decolorizing ability of the *Arcyria cinerea* was evaluated at different concentration of dye from 50 to 500 mg/L (Figure 5). The maximum colour removal of 96 % was observed at 78h in 50 mg/L Blue BB followed by 94% removal at 78 h in 100 mg/L. 89 % and 87 % dye removal was recorded at 84 h for 200 and 300 mg/L concentrations. However, a least removal of 70% was observed at 96 h for 500 mg/L. This shows that the high concentration of the dye has inhibitory effect on dye decolorization activity. There are some reports on the toxicity and genotoxicity of textile dyes and waste water[22,1,18].

3.3.5. Effect of age of the fungus

The age of the fungus seems to influence the decolorisation of Blue BB. In a series of batch experiments, *Arcyria cinerea* grown in sabouraud dextrose broth was transferred to fresh medium containing dye solution at the end of 1st, 2nd, 3rd, 4th and 5th days respectively. The 3rd day old fungus decolorized the dye most efficiently where nearly 96% of the dye was degraded (Figure 6). The 5th day old fungus did not show any significant decolorisation. Zhou and Banks[26] suggested that the resulting increase in adsorption with culture age was due to the increase in the cell wall mass.

4. CONCLUSION

Fungal decolourization is a recent alternative to replace the present chemical treatment processes. The present study reveals the inherent potential of *Arcyria cinerea* in the management of industrial dyes (removal 96.82%). The lesser explored organism *Arcyria cinerea* could emerge as a alternative time effective biocleaning system when grown in optimum conditions of 25°C temperature at pH 5 under 150 rpm at 78 hours. These findings can serve as an important base for the development of economical as well as simplified biological treatment.

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