International Biodeterioration & Biodegradation 77 (2013) 1–9

Contents lists available at SciVerse ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod

Contribution of lignin degrading enzymes in decolourisation and degradation of reactive textile dyes

Rina D. Koyani [a,*], Gaurav V. Sanghvi [a], Rakesh K. Sharma [b], Kishore S. Rajput [a]

[a] Department of Botany, Faculty of Science, The Maharaja Sayajirao University of Baroda, Vadodara 390002, India
[b] Department of Applied Chemistry, Faculty of Technology, The Maharaja Sayajirao University of Baroda, Vadodara 390002, India

ARTICLE INFO

Article history:
Received 31 July 2012
Received in revised form
6 October 2012
Accepted 12 October 2012
Available online 23 December 2012

Keywords:
Phanerochaete chrysosporium
Biodecolourisation
Biodegradation
Solid state fermentation
Ligninolytic enzymes

ABSTRACT

Investigating the potential of *Phanerochaete chrysosporium* to decolourise and degrade two reactive textile dyes (Reactive Yellow MERL and Reactive Red ME4BL) was the main intend of the study. The fungus analysed for its decolourising potential has shown the significant success by removing the colour of tested dyes (10 mg/L concentration) within 11 days of incubation. Supplementing media with different carbon/nitrogen sources proved dextrose and aspargine as efficient decolorizing enhancers, while inoculum size of 1 and 3 (10 mm diameter agar plug) were more supportive for solid and liquid decolourisation respectively. The ligninolytic enzyme production under solid state fermentation involved different agro-industrial wastes, among which wheat straw with 1 mm particle size was responsible for optimum production of manganese peroxidase (607.35 IU/ml), manganese independent peroxidase (539.27 IU/ml) and laccase (263.03 IU/ml). The partially purified enzymes produced by *P. chrysosporium* was achieved at four different percent saturations i.e. 20, 40, 60 and 80, where 60% saturated fraction produced the maximum 607.35 IU/ml of manganese peroxidase having 52.8 kDa molecular weight. The biodegradation of Reactive Yellow MERL (7-(4-{4-chloro-6-[3-(2-sulfoxy-ethanesulfonyl)-phenylamino]-[1,3,5]triazin-2-ylamino}-2-ureido-phenylazo)-naphthalene-1,3,6-trisulfonic acid) and Reactive Red ME4BL (5-{4-choloro-6-[4-(2-sulfo-ehtanesulfonyl)-phenylamino]-[1,3,5]triazin-2-ylamino}-3-(1,5-disulfo-napthalen-2-ylazo)-4-hydroxy-naphthalena-2,7-disulfonic acid) were studied by FTIR analysis where shifting of peaks confirmed the complete degradation of both the dyes.

1. Introduction

The Saurashtra region of Gujarat state, one of the most flourishing textile centres of the country (India), is a significant contributor for generating hazardous wastes and causing aquatic toxicity. The textile effluent using the water drainage system directly contaminates the canals, rivers and even ground water of the area. The effluent has been percolated at such an extent that water drained from the ground is colourful. This state of affairs is not only in the Saurashtra region, but it is also prevailing almost in all developing countries. Almost 7×10^5 tones of dyes are produced around the world every year, and most of them are azo dyes consisted of one or more azo groups (R1-N N-R2), which are extensively used as industrial raw materials (Meyer, 1981; Zollinger, 1987). The abundant enhancement of the dyes in the environment is causing a serious problem worldwide, owing to their incremental accumulation in the food chain and continued persistence in the ecosystem. With increasing environmental concerns, degradation of these textile dyes has become an area of intense research. Even though many physical (Vandevivere et al., 1998) and chemical (Jozwiak et al., 2007) processes have been and are being tried for removal of dyes, decolourisation and degradation using biological agents is promising, eco-friendly and cost-effective alternate.

However, many microorganisms have been found to decolorize and degrade undesirable/colorful discharges of the dyes, the potential of co-oxidization and transformation of various hazardous chemicals by fungi has aroused an interest in using them in biodegradation of dyes (Bosma et al., 2001). The ability of the white-rot fungi to degrade a wide range of recalcitrant dyes has generally been associated with the non-specific nature of their lignin modifying enzymes (Field et al., 1993; Goszczynski et al., 1994). Since the enzymatic system secreted by Basidiomycetes depends even on the strain and culture conditions, more number of white-rot fungi need to be screened for their ability to degrade xenobiotic compounds including dyes (Zouari-Mechichi et al.,

* Corresponding author. Tel.: +91 9428157816.
E-mail address: koyani_rina@yahoo.co.in (R.D. Koyani).

0964-8305/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.ibiod.2012.10.006

2006). Consequently in the present study, after screening of thirty five isolated strains, *Phanerochaete chrysosporium* as one of the potential isolate was selected and investigated for its decolourisation and degradation adequacy.

The production of ligninolytic enzymes depends on the mode of cultivation or fermentation, where solid state fermentation (SSF) is appropriate as it provides the natural living condition to fungi. In the SSF, use of agro-industrial residues promote the excellent growth of fungi and enhance the enzyme activity by means of supplying the nutrients to the fungi. Moreover, it also offers the possibility of reusing and managing the agro-industrial waste fruitfully. The key process parameter to be set for the excellent growth and enhanced enzyme production is the temperature; therefore, to provide natural environment the SSF was carried out at room temperature. Crude and partially purified enzyme was preferred for their application in enzyme assay and degradation, as the complete purification process presents high cost. In order to determine the optimum dye degradation, influence of carbon/nitrogen sources and inoculum size on dye decolourisation, and effect of pH, temperature, inoculum size, incubation and reaction time, and metal ions on enzyme productivity or activity was carried out. The intermediates formed during degradation of the dyes were analysed by FTIR (Fourier Transform Infrared Spectroscopy).

2. Materials and methods

2.1. Isolation and screening of the fungi

Thirty five strains of wood rot fungi (fungal fruiting bodies and infected wood samples) were collected from different forests viz. Junagarh (Saurashtra region), Pavagarh (Central Gujarat) and Waghai (Dangs forest from South Gujarat) of Gujarat State. Collected fruiting bodies and wood samples were surface sterilized by routine method of 0.1% $HgCl_2$ with an intermediate washing by sterile distilled water followed by a treatment of 70% ethanol. Finally, treated samples were inoculated on different media and incubated at room temperature (28–38 °C). Isolated rot fungi were purified and grown on optimized malt extract agar (MEA) medium. To screen the white rot fungi, purified cultures were subjected to Bavendamm's test (Bavendam, 1928) and among six screened white rot fungi, KSR-17 was considered for the present studies. Molecular identification of the strain was attained through Chromous Biotech Pvt. Ltd., Bangalore (India).

2.2. Chemicals and dyes

DMAB (3-dimethyl amino benzoic acid), MBTH (3-methyl-2-benzothioazolinone hydrazone hydro chloride), H_2O_2 and Manganese Sulphate ($MnSO_4$) were purchased from National chemicals Ltd., (India). Malt extract powder and electrophoresis chemicals were acquired from Himedia (India). The textile dyes used in the present study were kindly provided by the dying and printing houses i.e. Krishnakant textile industry and Deep text textile industry (Gujarat, India). Chemicals acquired for the biochemical studies were commercially available products of analytical grade.

2.3. Dye decolourization

2.3.1. Solid plate decolourization

Two reactive dyes (Reactive Yellow MERL and Reactive Red ME4BL) were subjected to solid plate decolourisation assessment on the Malt Extract Agar (MEA) plate containing 2% malt, 2.5% agar in the presence of individual dye. The media supplemented with dye concentrations (10, 50, 100, 250 and 500 mg/L) were poured into plates and inoculated centrally with 10 mm diameter agar disc removed from the actively growing fungi on Malt Extract Agar medium. The decolourisation efficiency was assessed by visual dye disappearance on the plates from 3rd to 15th day of inoculation. At every 2 days of interval, the zone of growth and decolourisation were measured. All the sets were performed in triplicates.

2.3.2. Decolourisation in liquid media

Liquid dye decolourisation was performed in 150 ml Erlenmeyer flasks containing 25 ml of Malt Extract Broth (MEB, 2%) supplemented with respective dyes (10 mg/L concentration). Three discs (10 mm diameter) from seven days old cultures of pure isolates were inoculated in each flask containing sterilized media. Inoculated flasks were harvested after 3, 5, 7, 9 and 11 days and filtered with whatman filter paper for the removal of mycelia. Dye decolourisation was monitored spectrophotometrically using filtrate at the maximum visible wavelength of absorbance (λ_{max}) for individual dyes. The decolourisation efficiency was expressed as per the following equation.

$$\%\ \text{decolourisation} = \frac{\text{Initial absorbance} - \text{observed absorbance}}{\text{Initial absorbance}} \times 100$$

All the experiments were performed in triplicates and the average values were considered in calculations.

2.3.3. Influence of carbon/nitrogen sources on decolourization

Various carbon (such as dextrose, sucrose, fructose, and lactose) and nitrogen (such as ammonium sulphate, sodium nitrite, asparagine, and urea) sources, at the concentration of 10 g/L were used as co-substrates to investigate their effects on decolourisation. Influence of these sources on the solid plate decolourisation was checked by inoculating the petri dishes (containing growth medium, dyes and carbon/nitrogen sources) with a disc (10 mm diameter) of fungal mycelium. The diameters (cm) of the decolourisation and growth zone were obtained in two perpendicular directions of the plates at the interval of every 2 days. Uninoculated plates containing dyes and carbon/nitrogen sources were treated as control.

While in liquid decolourisation, three plugs of fungal mycelia (10 mm diameter) were inoculated in the flask containing MEB (Malt Extract Broth) supplemented with dyes and carbon/nitrogen sources (1% concentration). Medium without any of the supplement was used as blank, whereas media with dyes and carbon/nitrogen sources but without inocula were used as control. Decolourisation efficiency of the fungal isolates was measured at the interval of every 3 days using UV-visible spectrophotometer (Perkin–Elmer, USA) and percent decolourisation was calculated as per above mentioned equation.

2.3.4. Influence of inoculum size on decolourisation

Malt extract agar plates containing respective dyes were inoculated with four different inoculum sizes (1–4, and 10 mm agar plugs) in order to assess effect of inoculum size on solid plate decolourisation. Growth and decolourisation zone were measured periodically from the 3rd to 9th day of inoculation to measure the decolourisation efficiency of fungus. However, role of inocula size in liquid decolourisation was assessed by inoculating flasks containing MEA-dye medium with different inoculum sizes (3, 6, 9, 12, and 15 of 10 mm diameter agar plugs). The filtered contents of all five flasks were subjected to spectrophotometric analysis after 5th day of incubation. Medium with dye but without inoculum was considered as control and medium containing only sterile media without any supplement was used as blank.