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Effect of pH and Nutrient Starvation on Biodegradation of Azo Dyes by *Phanerochaete chrysosporium*-Rp78

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Azodyes are potential chemical pollutants of industrial origin. The effect of pH and starvation of certain nutrients on biodegradation of reactive azodyes- Procion Brilliant Red H-E7B (Reactive Red 141), Procion Yellow H-E4 (Reactive yellow 84), Procion Navy Blue H-ER (Reactive blue 171) were studied. The high percentage of degradation was achieved at dye concentrations 12 mg L^{-1} for procion brilliant red i.e. 64.16%, 14 mg/l for procion navy blue and procion yellow dyes 76% and 57.14%respectively. The Nutrient limited media having 0.5% glucose, 0.06% Aspargine, 0.2% $MgSO_4 7H_2O$ and 0.5% KH_2Po4 at a pH-4.5 was found to be very effective for the degradation of selected dyes.

Key words: Reactive Azodyes, degradation Textile industry, Bioremediation.

Dyes are extensively used in the textile, paper, food, cosmetics and pharmaceutical industries (Chen *et al*., 1999). Azo dyes are poorly biodegradable because of their structure (Kim and Shoda, 1999) and, though they represent a potentially important class of organic pollutants, little is known about their fate in the environment (Chivukula and Renganathan, 1995). Treatment of water discharges containing dyes involves various physical/chemical methods viz., coagulation, precipitation, adsorption by activated charcoal, oxidation by ozone, ionizing radiation and ultrafilteration.These methods are not only costly but also generates wastes which are difficult to dispose of, less efficient and of limited application (Chen *et al*., 1999). Despite this, biodegradation of azo dyes has been reported using different microorganisms (McMullan *et al*., 2001), bacteria (Rajaguru *et al*., 2000), yeasts (Martins *et al*., 1999) and filamentous fungi, such as the white rot fungi (Martins *et al*., 2001; Pointing, 2001), *Proteus mirabilis* (Chen *et al*., 1999), *Pseudomonas* sp. (Oranusi and Ogugbue, 2001) *Pseudomonas luteola* (Hu,1998) and *Mycobacterium avium* (Jones *et al.*, 2003). Under anaerobic or microaerophilic conditions, azo dyes

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are degraded to aromatic amines by the enzyme azoreductase secreted by microorganisms (Plumb *et al*.,2001; Yoo *et al.,* 2001). These aromatic amines are toxic, mutagenic and carcinogenic (Bell *et al*., 2000) and are a potential source of concern in the environment. Under aerobic conditions, the aromatic amines are metabolised further, therefore, completing the detoxification of aromatic amines. Biodegradation of pollutants in natural ecosystems is influenced by various environmental factors including pH, temperature, salinity, cations, anions, biochemical oxygen demand, chemical oxygen demand and oxygen (Ganesh *et al.,* 1994). Biodegradation of dyes has been monitored using various indices: decolourisation (Chen *et al.,* 1999; Oranusi and Ogugbue, 2001); total viable count (Oranusi and Ogugbue, 2001) and mineralization (Blumel *et al.,* 1998). In the present study, we investigated the effect of pH and nutrient (nitrate and sulphate) starvation on biodegradation (decolourisation) of azo dyes such as Procion Brilliant Red H-E7B (Reactive Red 141) Procion Yellow H-E4 (Reactive yellow 84) Procion Navy Blue H-ER (Reactive blue 171) by *Phanerochaete crysosporium-Rp78* under aerobic conditions.

MATERIAL AND METHODS

Maintenance of organisms

The *phanerochaete crysosporium RP-78* was obtained from the Forest products laboratory, Madison, WI, and the cultures of *Aspergillus niger* was procured from Microbial type culture collection, Institute of Microbial Technology, Chandigarh, India. The pure cultures of *Phanerochete crysosporium R*p-78 was maintained in YMPG (Yeast Extract, Malt extract, Peptone, and Glucose) medium by serial sub culturing for every 15 days and stored at 4°C.

Dyes

The procion dyes nothing but the reactive azo dyes viz., Procion Brilliant Red H-E7B (Reactive Red 141), Procion Yellow H-E4 (Reactive yellow 84); Procion Navy Blue H-ER (Reactive blue 171) was procured from Atul Dyes Manufacturing Limited Gujarat, India.

Culture Media

In the present study the media with minimum concentration of nitrogen was used for the degradation. The composition of the medium is as follows: Glucose (10gm/l), Aspargine (1gm/l), KH_2PO_4 (2gm/l), $MgSO_47H_2O$ (1gm/l), pH-4.5)

Experimental procedure for dye decolourisation

The selected dyes were weighed precisely and aqueous stock solutions were prepared. The dye solutions were filter sterilized. The absorption maxima λ max for each dye was determined using spectrophotometer and the calibration curves were plotted.

Degradation studies

The experimental procedure for dye decolourisation was followed as per the method described by Colleen *et al*. (1990). The *Phanaerochete chrysosporium*-RP-78 lyophilized cultures were revived on YMPG medium. The degradation medium was prepared in Erlenmeyer flask and was sterilized in autoclave at 121^0C, 15 lbs pressure for 20 minutes. Then the media was inoculated with 2% overnight-developed seed culture of *Phanerochete chrysosporium*-RP78 grown at RT in an Orbital shaker at 200 rpm for a period of 6 days. On the sixth day, the dye was added to the medium. The absorption of zero hour sample i.e. the initial media containing the dye was taken by using the medium with out dye as blank. From the time of inoculation the samples were collected by centrifuging broth at 10000 rpm for 15 minutes and their absorbance was taken as wavelength maximum for each dye. The rate of degradation was determined by decrease in the absorption. After 5 days of dye inoculation some of the dye still adsorbed by the fungal mycelia. In an attempt to solubilize the bound dye if any, the mycelia was homogenized in 10 ml of methanol using homogenizer. The homogenate was centrifuged at 10000 rpm for 15 minutes and the mycelia pellet was suspended in an additional 5 ml of methanol and re-centrifuged and both the supernatants were combined. The absorbance of the supernatant was measured by using the methanol as blank and the amount of dye associated with the mycelia was calculated from standard graph. The percentage of degradation was calculated by using following formula.

$$\text{Percentage of degradation} = \frac{\text{Initial concentration - Final concentration}}{\text{Initial Concentration}} \times 100$$

Optimization of degradation of dyes

Dye concentration

Influence of concentration of dye on degradation was investigated by adding different concentrations of dye in to the medium ranging between 6 to 12 mg/liter for procion brilliant red and 10 to 18 mg/liter for procion navy blue and procion yellow. Then the rate of degradation of the dyes was determined following the procedure mentioned above.

Carbon source

To determine the influence of carbon source on degradation of dyes was studied by taking different concentrations of glucose in the medium varying between 0.5-2.5% (0.5%, 1.0%, 1.5%, 2.0%, and 2.5%). After adding the glucose in to the medium, the medium was sterilized and inoculated with mycelium and incubated for 6 days. The rate of degradation of the dyes was determined using the similar procedure mentioned earlier.

Nitrogen source

In general concentration of nitrogen shows significant effect on dye degradation. In the present study L-Asparagine was taken as nitrogen source. To know the requirement of optimum nitrogen concentration, different concentrations of L-Asparagine (0.06%, 0.08%, 0.1%, 0.12% and 0.14%) was maintained in the medium. The medium was inoculated with mycelium and incubated for six days. On the sixth day, dye was added to the medium and the rate of degradation was studied.

KH_2PO_4 concentration

Influence of KH_2PO_4 concentration on degradation was investigated by taking different concentrations of KH_2PO_4 varying from 0.05%, 0.1%, 0.15%, 0.2% and 0.25%. After adding the different concentrations of KH_2PO_4 in to the medium, it was sterilized, inoculated with mycelium and incubated for 6 days. The rate of degradation after sixth day was observed by monitoring the absorbance in the medium.

$MgSO_4\,7H_2O$ concentration

The effect of $MgSO_4\,7H_2O$ on degradation was studied by using the different concentrations of $MgSO_4\,7H_2O$ (0.05%, 0.1%, 0.15%, 0.2% and 0.25%). The rate of degradation was studied similarly as mentioned above.

Optimization of pH

To know the optimum pH for the dye degradation, the pH of the medium was varied from 3 to 6. And the values were adjusted and maintained with 0.01N HCl.

RESULTS AND DISCUSSION

The absorption spectra of the three selected procion dyes were shown in fig. 1-3. The absorption maximum (λ_{max}) of procion navy blue, procion yellow and procion brilliant red was observed to be 610 nm, 408 nm and 543 nm respectively. For biodegradation studies, and to determine the ability of *Phanerochaete chrysosporium*-RP78 in decolorizing the selected dyes, the experiments were performed. The results indicate that the ability of *P. chrysosporium* was varied. In all the experiments, most of the colour loss occurred within three days; however some of the dye remains adsorbed to the mycelia that can be desorbed with methanol after five days. Among the three dyes, the rate of degradation of the procion navy blue was found to be very high followed by brilliant red; nevertheless the procion yellow was not completely degraded by *P. chrysosporium*. Various physico-chemical parameters were optimized for obtaining effective degradation of dyes.

Dye concentration

To obtain maximum degradation, experiments with different initial dye concentrations were performed. Results clearly show that the process efficiency in terms of percent decolorization and treatment capacity was affected by dye concentration. Spectroscopic examination of methanol extracts of the fungal mats after 5 days of incubation with a dye showed that the degradation ability was increased up to a particular concentration beyond that there is no considerable degradation. The high percentage of degradation, 64.16% was obtained at dye concentrations 12 mg/l for procion brilliant red, 76%, and 57.14% at concentration of 14 mg/l for procion navy blue and procion yellow dyes respectively (Figure-4-6). Similar results were obtained in the optimization studies of dye degradation by Mielgo *et al*. (2003) using Manganese peroxidase. A possible explanation for

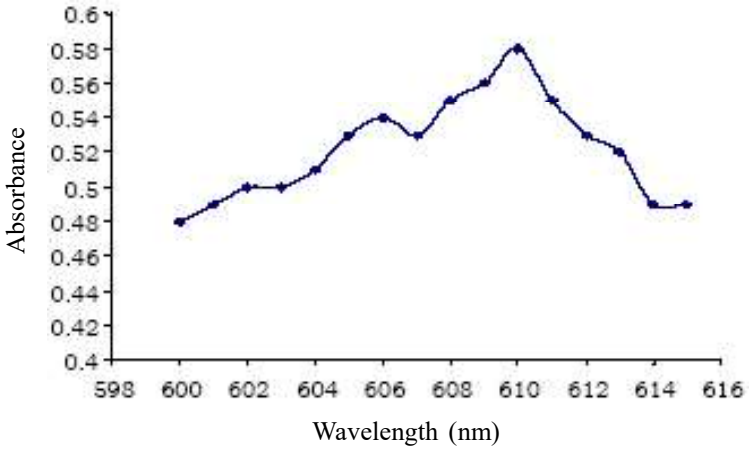

Fig. 1. Absorption spectra of procion navy blue

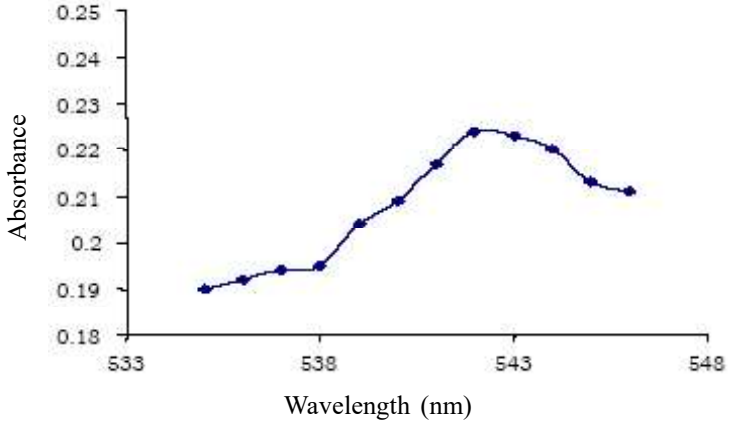

Fig. 2. Absorption spectra of procion brilliant red

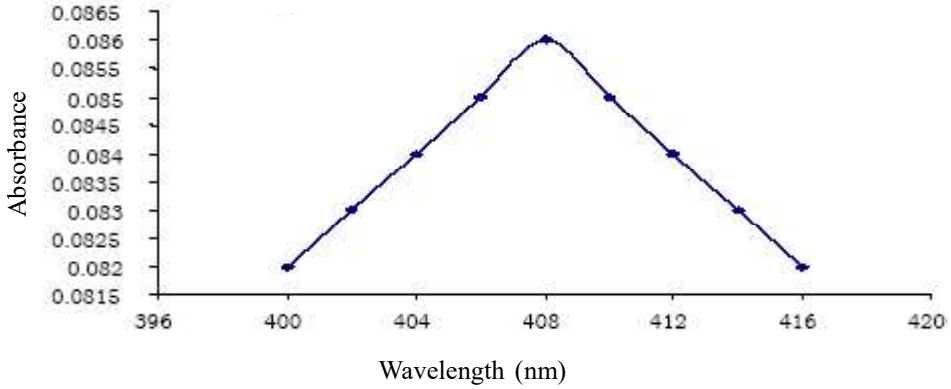

Fig. 3. Absorption spectra of procion yellow

this could be the saturation of the enzyme in the presence of high concentrations of dye and the product inhibition by the dye itself or its derivatives might have occurred by binding to the active site of the enzyme.

Carbon and Nitrogen source

It was well documented that the concentration of the carbon and nitrogen source are the major determining factors for designing an effective degradative system (Adosinda *et al*., 2001). Cells grown in high nitrogen and carbon source were reported to be less effective in dye degradation than the cells grown in the low nitrogen and carbon containing medium (Glen and Gold, 1983) because it is possible that the lignin degradative system which is also involved

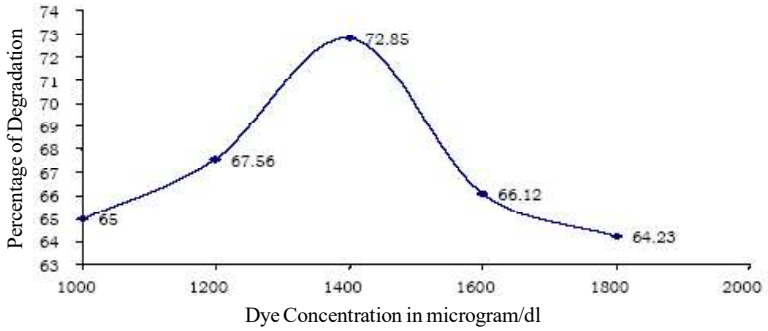

Fig. 4. Optimization of dye concentration for procion navy blue degradation

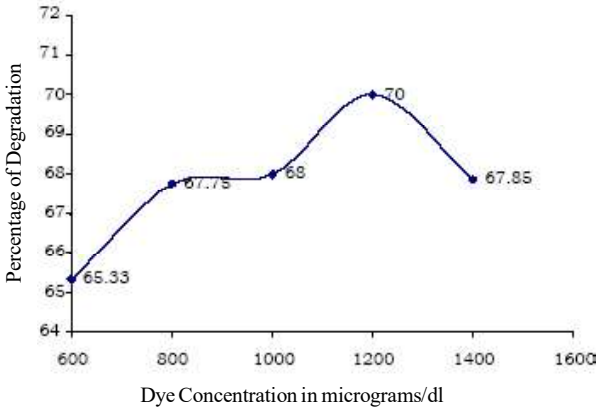

Fig. 5. Optimization of dye concentration for procion brilliant red degradation

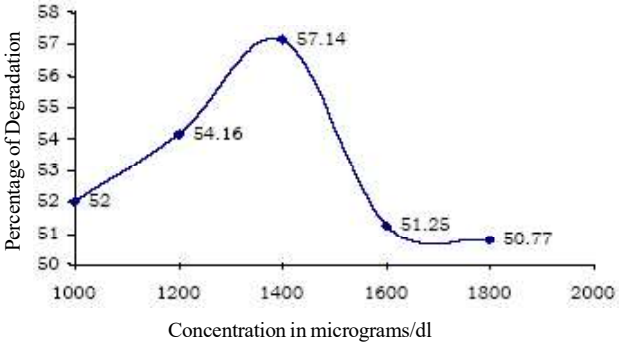

Fig. 6. Optimization of dye concentration for procion yellow degradation

in the dye degradation is highly expressed in the nutrient limiting conditions. In consonance with this, a high decolourization of 62% for procion yellow, 76% for procion navy blue and 70% for procion brilliant red was observed at a concentration of 0.5% glucose (Fig.7). In addition, 70.85% procion yellow, 82% procion navy blue and 74% procion brilliant red decolouruzation was observed at a concentration of 0.06% aspargine (Fig. 8).

Magnesium concentration

Taking into account that the $MgSO_4 7H_2O$ can directly affects the catalytic properties of lignin peroxidase was determined

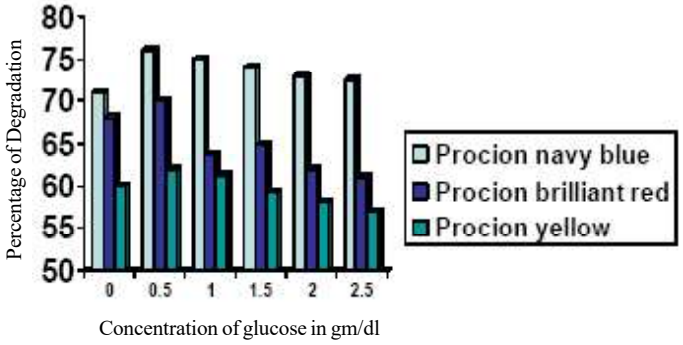

Fig. 7. Optimisation of dye concentration for procion for dye degradation

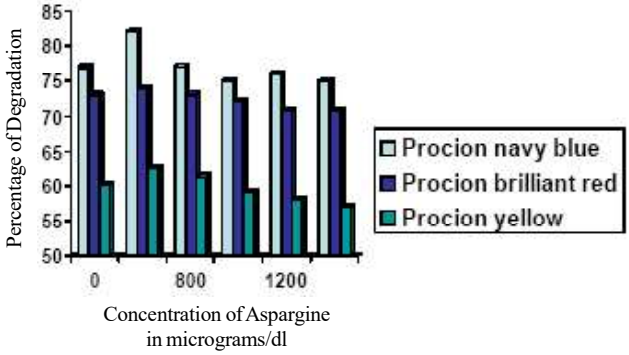

Fig. 8. Optimisation of Nitrogen concentration for dye degradation

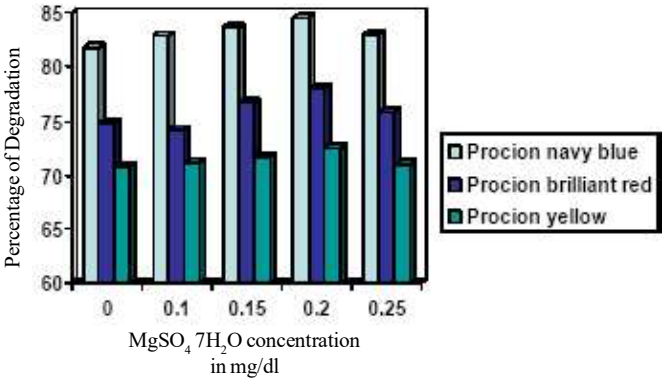

Fig. 9. Optimisation of $MgSO_4 7H_2O$ concentration for dye degradation

by taking different concentrations of magnesium (0.05 to 0.25 mg/l). The results envisaged that the percentage of degradation was increased with the increase of magnesium up to 0.2% and there after a decrement in degradation percentage was observed (Fig. 9). The maximum decolorization i.e. 84% for procion navy blue, 78% for procion brilliant red and 72% for procion yellow were obtained at 0.2% concentration of $MgSO_4 7H_2O$.

Potassium concentration

Similar to magnesium, the requirement of KH_2PO_4 concentration in smaller quantities is well demonstrated that it can also affect the decolourisation of the dyes up to a concentration of 0.5%. Beyond this concentration, it has not showed any effect. A maximum decolorization of 88% was seen with procion navy blue followed by procion brilliant red (80%) and procion yellow (74%) respectively (Fig. 10). A possible explanation is that K^+ is an important factor required for the lignin degradative system in bioremediation at only in lower concentrations. In contrast to the observations of present study, KH_2PO_4 and $MgSO_4$ were unable to show any influence neither on the decolurization of kraft pulp effluents employing *Rhizopus oryzae* nor on the general growth of the organisms (Wuye *et al*., 2003) there by indicating that the mechanism involved in our study is dependent on Mg and K ions for the degradation process.

Optimization of pH

To know whether the pH shift would have any influence on decolorization, experiments were conducted at varied pH (3.0 to 9.0). The results indicate that the maximum degradation i.e. 90% for procion navy blue, 83% procion brilliant red and 76.42% for procion yellow was obtained at pH 4.5 when compared with higher pH range (Figure-11). It was reported that for practical application of any enzyme in the process of decolorization conditions closer to industrial operation, higher temperatures and less acidic pH conditions required (Mielgo *et al.,* 2003).

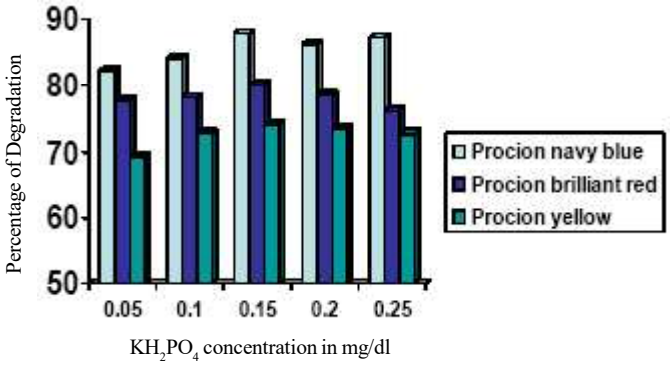

Fig. 10. Optimisation of KH_2PO_4 concentration for dye degradation

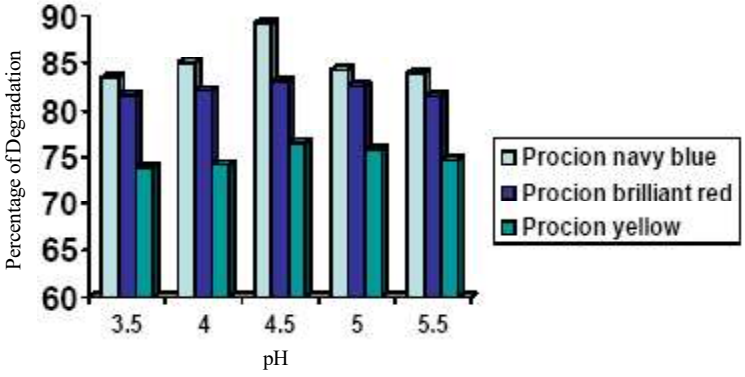

Fig. 11. Optimisation of pG for dye degradation

CONCLUSION

The present study envisaged that the genome sequenced *Phanerochaete chrysosporium*-RP78 was observed to be an effective fast degrader organism for decolourization activity on selected textile reactive azo (Procion) dyes, and the N-limited media having 0.5% glucose, 0.06% Aspargine, 0.2% $MgSO_4 7H_2O$ and 0.5% KH_2PO_4 at a pH-4.5 was found to be very effective for the degradation of selected dyes. .

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