Decolourization of Reactive azo dyes by *Aspergillus niger* from dying industry effluent

Rizwana Parvez. S and Uma Maheswari Devi .P
Department of Applied Microbiology
Sri Padmavati Mahila Visvavidyalayam,Tirupati-517502, A.P., India.
Email of corresponding author: umadevi66@yahoo.co.in

Abstract— The textile industry, which is one of the largest water consumers in the world, produces wastewater composed of various recalcitrant agents such as dye, sizing agents, and dyeing aids, that should be of concern in releasing into the environment. The present study was carried out to examine the fungal decolourization of hazardous azo dyes by utilizing single fungus *i.e.*, *Aspergillus niger* as the experimental organism and selected textile azo dyes Orange107 and Red 1.The apparent dye removal for dyes was seen largely due to biosorption/bioadsorption into/onto the fungal biomass. Decolourization capabilities of these fungal species against the azo dyes were carried out in potato dextrose agar medium and Mineral salt media under static invitro conditions and different physic-chemical conditions on dye decolourization were studied. Highest percentage of decolourization was achieved against Orange-107 by *Aspergillus niger* (92%) after 5 days of incubation when compared to Red -1 dye. This study has confirmed that the potential fungi *Aspergillus niger* in the decolourization of azo dyes and opened scope for the future analysis of their performance in the treatment of textile dyes.

Index Terms— *Aspergillus sps*, Decolourization, Orange107, Plate assay method, Red 1

1 INTRODUCTION

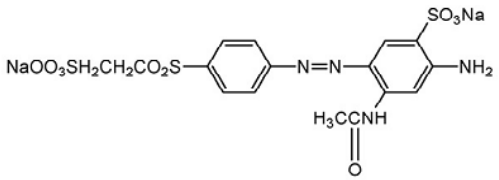
Coloured wastewater from textile industry is rated as the most polluted in all industrial sectors. It has been estimated that over 10,000 different textile dyes and pigments are in common use and the total world organic colorant production is more than 100,000 tons/year [1][2][3]. Based on Chemical structure of chromophore there are 20 -30 different groups of dyes. Azo (Monoazo, diazo, triazo, polyazo), anthraquinone, phthalocyanine and triarylmethane dyes are the most important groups [4]. Azo dyes are the largest class of synthetic aromatic dyes composed with one or more (N=N) groups and sulfonic (-SO3-) groups with lots of commercial interest [5][6]. Azo dyes are water-soluble synthetic organic compounds. Generally, azo dyes contain one, two or three azo linkages, linking phenyl, naphthyl rings that are usually substituted with some functional groups including triazine amine, chloro, hydroxyl, methyl, nitro, and sulphonate [7]. About 80% of azo dyes are used in the dyeing process of textile industries. It had been estimated that approximately 10% of the dyes used in dying process do not bind to the fiber and are released into the environment [8]. They possess toxicity like lethal effect, genotoxicity, mutagenicity, and carcinogenicity to plants and animals [9].Synthetic dyes specially, sulfonated and their related biodegradation products contain structural elements, which are unknown or rare in nature; they not only have a negative aesthetic effect but also resist microbial attack and contribute to aquatic and soil toxicity[10][11][12][13][14].The decolourization of textile waste water is still a major environmental concern because of synthetic dyes which are difficult to be removedby conventional treatment systems [15][16][17].Commonly applied treatment methods for colour removal from coloured effluents consist of integrated processes involving various combinations of biological, physical and chemical decolourization methods [18][15][19]. Chemical and physical methods for treatment of dye wastewater are not widely applied to textile industries because of exorbitant costs and disposal problems. Bioremeiation is an alternative starategy to deal with this problem include adsorption of dyestuffs on bacterial and fungal biomass [20][21] or low-cost non-conventional adsorbents[22][23].

2 MATERIALS AND METHODS

2.1 Dyes

Azo dyes- Reactive Orange 107 (RY107) and Reactive Red-1 is a Reactive vinyl sulphone dyes collected from Madanapalle, Chittoor district, Andhra Pradesh, India.Name: C.I. Reactive Orange 107; Molecular Structure: Single azo class; Molecular Formula: C16H16N4Na2O10S3; Molecular Weight: 566.49 as shown in Fig 1.

• *Corresponding author: Prof. P Uma Maheswari Devi, Head, Department of Microbiology, Sri Padmavati Mahila isvavidyalayam,Tirupati,517502,Andra Pradesh,India. Ph:9848894900. Email: umadvi66@yahoo.co.i*



Name: C.I.Reactive Red 1; Molecular Structure: Single azo class; Molecular Formula: $C_{19}H_9Cl_2N_6Na_3O_{10}S_3$; Molecular Weight: 717.38 as shown in Fig 2.

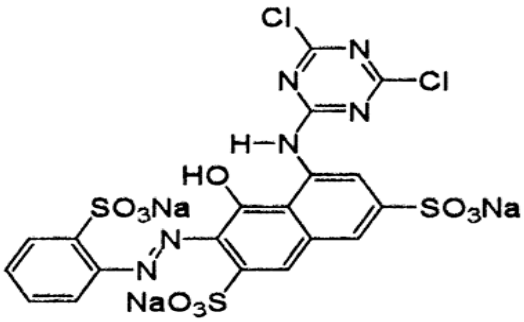


Figure 2: C.I. Reactive Red –1

2.2 Preparation of dye Stock Solution

Each dye is prepared by dissolving 2.0g in 10ml distilled water (200 mg/m1) sterilized by autoclaving at 110°C for 30 minutes.

2.3 Culture media

Potato Dextrose Agar (PDA) medium and Mineral salt media (MSM) used as selective media for growth of fungal strains for decolourization of dyes respectively.

2.4 Preparation of fungal inoculums

Aspergillus niger was grown in PDB on a rotator shaking incubator (100 rpm, 28°C) for 8 days and its biomass was then filtered and washed twice with distilled water. Fungal biomass (pellets) 5 g 100 ml–1 of distilled water was homogenized in a blender for 5 min and later used as inoculum in the experiments.

2.5 Dye-Agar plate Assay

The isolated fungi were screened for decolourization studies by Dye-agar plate assay.

2.6 Effect of physicochemical conditions on the decolourization

2.6.1 Effect of pH on dye decolourization

The effect of pH on dye decolourization was studied by varying the pH range from (3.0, 5.0, 7.0 and 9.0). The experiment was performed at 400 mg/L dye concentration.

2.6.2 Effect of temperature on dye decolourization

The effect of temperature on dye decolourization on Reactive Orange 107 (RY107) and Reactive Red-1 was studied by varying the temperature from (4°C, 27°C and 37°C). The experiment was performed at 400 mg/L dye concentration.

2.6.3 Effect of C and N sources

To study the effect of different carbon (glucose, sucrose and fructose) and nitrogen sources (1%) such as (Beef extract, Yeast extract and Peptone) were added as supplement individually to the Mineral salt medium for decolourization of both Reactive Orange 107 (RY107) and Reactive Red-1 dyes.

2.6.4 Decolourization percentage

To etermine the ecolourisation percentage,the red dye was incubated with A,niger for ifferent time intervals at their respective pH, temperature, 1% of Carbon and nitrogen sources and samples were removed at regular intervals and analyzed for decolourization activity. The percent decolourization of effluent was determined by using the formula.

$$D = \left[\frac{A_0 - A_1}{A_0} \right] X\,100$$

D = Decolourization
A_0 = Initial Absorption
A_1 = Final Absorption

2.7 Statistical analysis

The experiment was done in triplicate for each parameter. The results were expressed as percentage of decolourization with respect to control values. The Data were entered in Microsoft excel 2007 spread sheet an the 'mean & standard deviation' were calculated.

3 RESULTS AND DISCUSSION

In the present study the fungi was studied for the removal of orange 107 and Red-1 from aqueous solution. The results were tabulated in Figure 8. The fungus *Aspergillus niger* removed 92.0 per cent of dye from the aqueous solution within fourth day of incubation period, mycelial growth also increased up to 54.0 mg on fourth day.

3.1 Properties of C.I. Reactive Orange 107 (RY107)

Figure 1: C.I. Reactive Orange 107 (RY107)

3.2 C.I. Reactive Red-1

3.3 Plate Assay

Fungi mycelia agar disc (~2 mm) were cut from the colony margin (actively growing region) and inoculated on canter of petri dish (in triplicates) containing PDA supplemented with 400 mg of Reactive red- 1 dye was incubated at room temperature (~28° C) in dark for 7 days and un-inoculated dye agar plates were maintained as control. The size of the halo clearing indicates decolourization and was measured in two perpendicular directions of the plate Fig. 3.

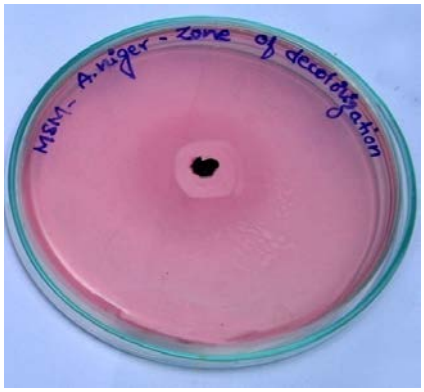


Figure 3: Zone of decolorization by *Aspergillus niger*

3.4 UV–VIS analysis

Decolourization activity was performed in 100 ml of MSM media with 400mg/L of dye concentration and inoculated with 10% (V/V) inoculum and incubated for different time intervals under static conditions. Un-inoculated dye medium served as control. After incubation, about 5ml decolourized samples were withdrawn aseptically at regular intervals and centrifuged at 10,000 rpm for 10 minutes. The clear supernatant was used for measuring absorption for RY-107 at 410 nm and for RR-1 at 540nm using UV-VIS spectrophotometer (UV-VIS-1601 SHIMADZU).

Figure 4: Bio-decolourisation of organge 107 by A. niger

Figure 4: Bio-decolourisation of Red 1 by A. niger.

3.5 Effect of pH and temperature

The medium pH is also important factor for better decolourization activity. Decolourization was observed in the pH range from 5 to 9 [24]. Similarly, for RY107 shows (91%) while RR-1 shows (84%) of decolourization at pH 7.0 when compared to other pH 3,5 and 9 as shown in Fig 6. In many studies, it was observed that the optimum pH for colour removal is often at a neutral pH or at slightly alkaline pH. The rate of colour removal was higher at only optimum pH but tends to decrease rapidly at strongly acid or strongly alkaline pH. Biological reduction of the azo bond can result in an increase in the pH due to the formation of aromatic amine metabolites, which are more basic than the original azo compound [25]. Altering the pH within a range of 7.0 to 9.5 has very little effect on the dye reduction process.[26] found that the dye reduction rate increased nearly 2.5-fold as the pH was raised from 5.0 to 7.0, while the rate became insensitive to pH in the range of 7.0–9.5.Both azo dyes RY107 shows (92%) while RR-1 shows (83%) of decolourization at 37°C temperature compared to 5 and 27°C as shown in Fig 7.It was observed that the temperature required to produce the maximum rate of colour removal tends to correspond with the optimum cell culture growth temperature of 35–45°C. The decline in colour removal activity at higher temperatures can be attributed to the loss of cell viability or due to the denaturation of an azo reductase enzyme [26] [27] [28].

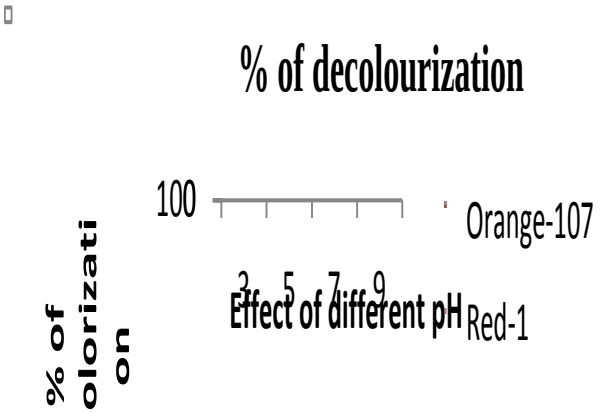


Figure 6: Effect of pH on decolourization of azo dyes

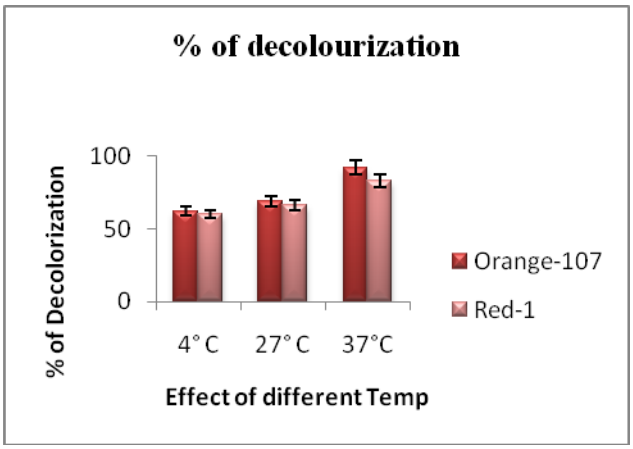


Figure 7: Effect of temperature on decolourization of azo dyes

3.6 Effect of Carbon and Nitrogen sources

To explore carbon effect, experiments were performed with different carbon sources (maltose, glucose, sucrose and fructose) keeping other conditions constant (pH, temp and N source). Maximum decolourization was observed with 1% glucose. To explore nitrogen sources effect, experiments were performed with different nitrogen sources (Beef extract, peptone and yeast extract) keeping other conditions constant (pH, temp and C source). Maximum degradation was observed with 1% yeast extract. The culture media supplemented with additional source of carbon exerted improvement both in the growth and degradation ability of *Aspergillus niger*.The addition of nitrogen sources was observed to have significant effect on degradation of the dyes Red1 and orange107 as shown in Fig 8. Mineral salt media supplemented with Yeast extract showed a high degree of degradation on both Red 1 and Orange107.This increased growth rate is attributed to enhanced bi absorption capacity and hence earlier decolourization by the fungus.

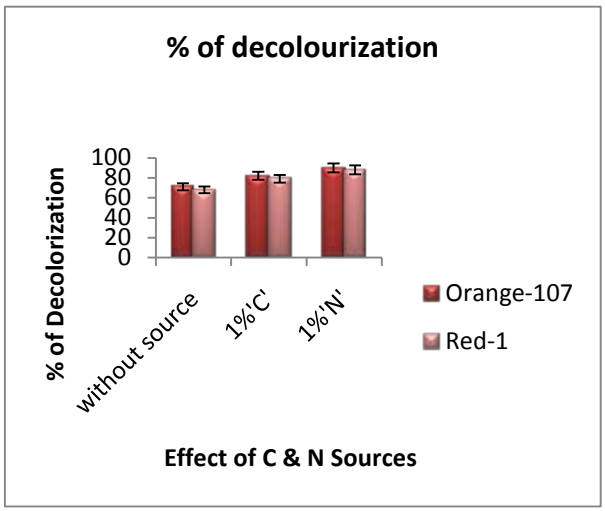


Figure 8: Effect of different C and N sources on decolourization of azo dyes

3.7 Decolourization assay

Decolourization of azo dyes reactive Red1 and orange107 under optimum conditions such as pH, temperature, carbon and nitrogen sources *Aspergillus niger* shows 92% in orange107 while in Red1 shows 88% of decolourization after six days of incubation Fig 9.

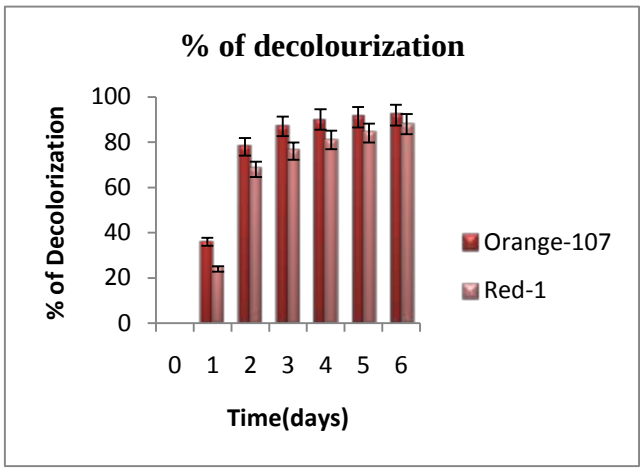


Figure 9: Effect of incubation time on percentage of decolourisation by *A. niger*.

4 Conclusion

A. niger strains appear to be an attractive option for the treatment of industrial dyes. *Aspergillus niger* can be used for the treatment of textile dyes and can be performed in low cost at the industrial site as compared to the anaerobic treatment which requires large input. To conclude, the decolourization of dyes was studied under optimum conditions, encouraging results were obtained after 3 days, but maximum decolourization of the dyes was obtained after 6 days. In this study we have observed higher decolourization with 1% 'N' source (Yeast extract) temp 37°C, pH-7 by *Aspergillus niger*.

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