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Decolorization of Symmetrical and Unsymmetrical 3-Nitroformazans using *Coriolus Versicolor*

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Abstract: Natural as well as synthetic dyes are well known and used for dyeing of various materials. Most common use of dyestuffs is in textile industry. Proper decolourisation and disposal of waste water from dyeing industry is an important issue. Symmetrical nitroformazans constitute a special class of azo-hydrazone dyes which have found application in dyeing industry. These dyes are a major cause of water pollution and need to be removed or destroyed before the disposal of waste water from dyestuff industry. Several methods like physical, chemical and biological have been reported for removal of dye in waste water. The commonly used physico-chemical techniques are costly, less efficient and are liable to interference by other waste water constituents. In the present study fungus, *Coriolus versicolor* was used for decolourisation of 3-nitro-1,5-diarylformazans. Maximum decolourisation of dye was observed after 96 hours incubation and minimum after 24 hour incubation.

Keywords: Azo dyes, formazans, waste water, biological decolourisation.

1. INTRODUCTION

Many natural dyes have been known for a long time. They were obtained from animal and vegetable sources. Organic compounds have an ability to impart their colour to the material to be dyed, in an aqueous medium. 3-nitro-1,5-diarylformazans, a special class of formazan dyes are azo-hydrazo compounds commonly used for dyeing purpose. Synthetic dyes are widely produced and used in many different industries including the textile, cosmetic, paper, leather and pharmaceutical and food.

Moreover, these industries consume substantial volumes of water and chemical products associated with the dyeing process. More than 10,000 different textile dyes and estimated annual production of 8×10^5 metric tonnes are commercially available worldwide, and about 50% of these are azo dyes [1,2].

The textile industry accounts for two thirds of the total dye stuff market (Riu et. al. 1998)[3] consuming a large quantity of reactive azo dyes with azo-based chromophores due to high demand for cotton fabrics with brilliant colour (Peternal et. al. 2006)[4].

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Azo dyes are characterized by their typical nitrogen to nitrogen (-N=N-) bonds. They are extremely versatile colorants and constitute about 50% of dyes produced [5]. Some of these dyes and/ or products of dye degradation are proved to be carcinogenic mutagenic and toxic[6,7]. Apart from the aesthetic deterioration of natural water bodies, dyes also cause harm to the flora and fauna in the natural environment.

Various physico chemical, advanced oxidation processes, biological process and usually a combination of processes are applied to treat the azo dyes containing waste water to meet regulatory discharge limits [8].

However, azo dyes due to their hydrophilic property are not removed by conventional methods. Further, the conventional waste water treatment has been found to be ineffective to solve the problem caused by release to the environment of coloured synthetic dye industrial effluents. The complex aromatic structure of the dyes is resistant to light, biological activity, ozone and other degradative environmental conditions[9].

Alternative methods, physical, chemical and biological have been proposed for the removal of dye in waste water[10,11]. The commonly used physico chemical techniques present some drawbacks such as high cost, low efficiency, limited versatility, interference by other waste water constituents and the need to handle the resulting waste and the advanced oxidation process are costly in terms of installation, operation and maintenance cost. On the other hand microbial decolourization appears as an attractive cost-effective option. Investment costs for biological process is five to twenty times less than chemical ones such as ozone or hydrogen peroxide and the running cost are three to ten times less [12]. Azo dyes are poorly biodegradable because of their structure[13] and though they represent a potential class of organic pollutant, little is known about their fate. Because of limitations of the conventional methods used for the degradation of azo dyes, the scientists have shifted their approach towards the use of conventional biological methods to treat waste water containing azo dyes. The metabolites produced after biodegradation are mostly non-toxic or comparatively less toxic in nature. Under anaerobic or micro aerophilic condition azo dyes are degraded to aromatic amines by the enzyme azo reductase secreted by micro-organisms [14] and are a potential source of concern in the environment.

Bacterial degradation of azo dyes is often mediated by azo reductases, which are more efficient under static and anoxic condition [15]. Similarly, lignolytic enzymes secreted extracellularly by fungal strains also produce higher decolourization of azo dyes by bacterial as well as fungal culture [16,17] results in more complete degradation and avoids accumulation intermediates. Various authors [18,19] emphasized the necessity of involvement of azo reductase cytoplasmic enzyme in the decolourization of azo dyes, often assuming electron

carriers (coenzymes). Wuhrmann et al proposed an intracellular chemical reduction of azo dyes by reduced Flavin nucleotides ($FADH_2$) [20]. Although decolourization may result from chemical reduction with reduced coenzymes, the coenzymes depend on cytoplasmic reducing enzymes to supply electrons. Gingell and Walker[21] who regarded the soluble Flavin as an electron shuttle between a dye and a NADH dependent azo reductase. Immobilization of microbial cells has received increasing interest in field of waste water treatment[22]. Immobilized cell system have the potential to degrade toxic chemical faster than conventional waste water treatment systems since high densities of specialized micro-organisms are used in immobilized cell systems. A considerable interest has been generated in studying microbial azo dye degradation[23]. In addition to the comprehensively studied white-rot fungi, several Bacillus species have been reported as azo dye decolourizers[24-27].

Castellaniella denitrificans SA13P is used as a novel strain for dye decolourization of malachite green [28]. *P. putida* cells (18 Engineered) were applied to decolourize the anthraquinone dye Acid Green (AG) 25 and diazo-dye Acid Red (AR). The results showed that decolourization of both dyes is Cu^{2+}- and mediator-independent, with an optimum temperature of 35ºC and pH of 3.0, and can be stably performed across a temperature range of 15ºC to 45ºC. A high activity toward AG25 (1g/l) with relative decolourization values of 91.2% (3h) and 97.1% (18h), as well as high activity to AR18 (1g/l) by 80.5% (3h) and 89.0% (18h), was recorded [29].

Namdhari et al [30], reported that under static *in vitro* condition the decolourization capabilities of the fungal species were evaluated for reactive blue MR dye (100-300 mg/L) in carbon limited Czapek Dox Broth (0.5%) were carried out. It was found that *A. allhabadii and A. sulphureus* showed higher decolouraization capabilities (95.13$\pm$0.11%), (93.01$\pm$0.25%) with 200mg/L dye, but *A. niger* showed higher decolourization (83.14%$\pm$0.19%) with 100mg/L after 10 days of incubation. Selvam and Shanmuga Priya et al [31] interpreted that by both batch mode and continuous mode the colour removal by the *Basidiomycetes* fungi was due to adsorption of the dyes to the mycelial surface and metabolic breakdown. The results also suggested that *Schizophyllum commune* was more efficient than *Lenzitesiemia* for the treatment of azo dyes.

Compared to other than suspension cultures, immobilized cultures tend to have a higher level of activity and are more resilient to environmental perturbations, such as pH or exposure to toxic chemical concentrations[32].

From above cited literature it is quite clear that among micro-organisms, bacteria are most commonly used for various bio remediation processes; as far as fungi are concerned their reports on bio remediation of dyes by fungi

are scanty. In the environment there are many micro-organisms which are abundant but their potential is not utilized properly.

In natural environment, azo dyes can be transformed or degraded by a variety of micro-organisms including aerobic and anaerobic bacteria and fungi[33]. Immobilization of microbial cells has received increasing interest in the field of waste water treatment [34,22]. Immobilized cell systems have the potential to degrade toxic chemicals faster than conventional waste water treatment system since high densities of specialized micro-organisms are used in immobilized cell systems.

In the present study an attempt has been made to assess the potential of the fungus *Coriolus versisolor* for decolourization and colour removal of 3-nitro-1,5-diarylformazans by using immobilized cells of *Coriolus versicolor* in a batch reactor.

2. DECOLOURIZATION OF DYES

The decolourization of Azo dyes was carried out as B. Chirsabesan and P. Mullai in a modified manner.

The medium used for dye degradation was yeast glucose agar medium of the following composition:

Table 1

Content	Composition (g/L)
Yeast Extract	5
Glucose	10
Agar	3%

The Fungal strain *Coriolus versicolor* was obtained from MTCC Chandigarh and the fungal culture was maintained on *yeast glucose agar medium.* Then the cells of *Coriolus versicolor* were sub-cultured and the culture was centrifuged at 10,000 rpm for 10 minutes. The Supernatant was discarded and the pellet was suspended in 3% sodium alginate slurry for immobilization. The slurry was dropped down drop wise into 75M chilled calcium chloride solution. The beads were then kept in calcium chloride for one hour at room temperature. The various concentrations (20%, 40%, 60%, 80%, 100%) of 3-nitro-1,5-di-m-tolylformazan were prepared in DMSO. In the test tube one ml of each concentration of dye was taken and then 10 beads per test tube were added.

The test tubes were incubated for different time period (0, 24, 48, 72, 96, 120h) at temperature 30ºC and pH=7.0.

The decolourization of dyes was measured by using spectrophotometer at 450nm and decolourization rate was expressed as percentage of decolourization calculated using the formula[35].

$$\text{Dye removal (\%)} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

3. RESULT OF DYE DEGRADATION

The decolourization of 3-nitro-1,5-di-m-tolylformazan at various concentrations like 20%, 40%, 60%, 80%, 100%, by using immobilized cells of *Coriolus versicolor* with different incubation time of 0, 24, 48, 72, 96 and 120h were observed.

The result of dye decolourization is given in Figure 7, 8, 9, 10, 11.

The decolourization was maximum after 96h and minimum at 24h incubation period in all concentrations. Among the different concentrations of the dye 20% recorded the maximum decolourization, and the minimum decolourization of dye occurred in 100% at 24h incubation period.

After 96h dye degradation process stopped and the results remained same at 120h incubation period.

So *Coriolus versicolor* has ability to decolourize this formazan dye.

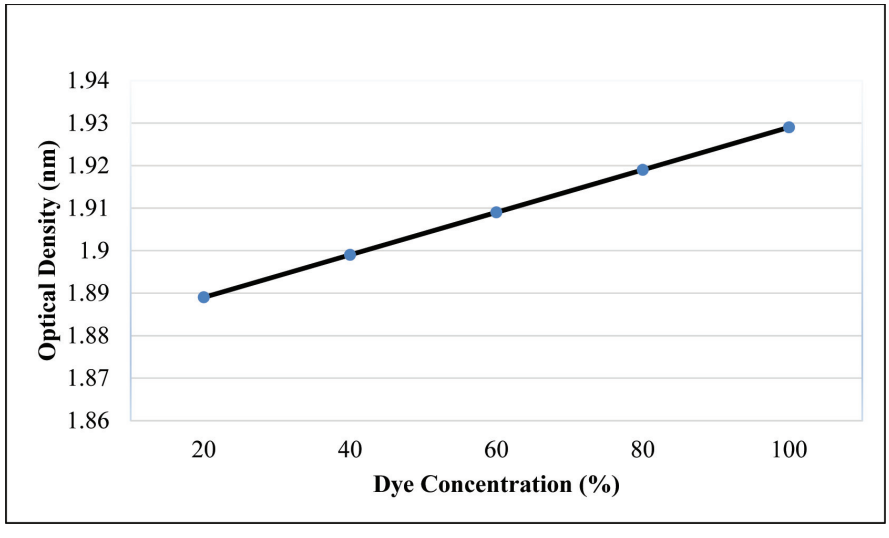

Figure 1: Initial optical density at 450 mm for different concentration of 3-Nitro-1,5-di-m-to1y1 formazan

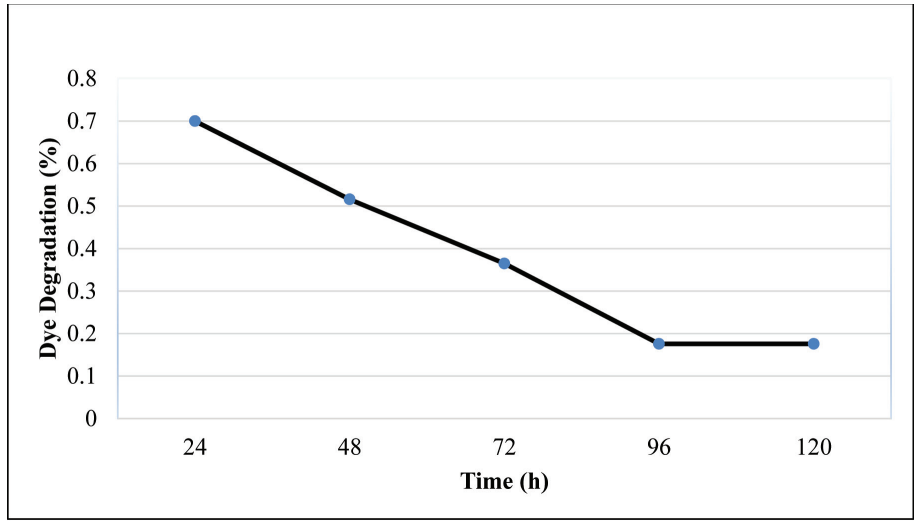

Figure 2: Decolourization of 20% 3-Nitro-1,5-di-m-tolylformazan

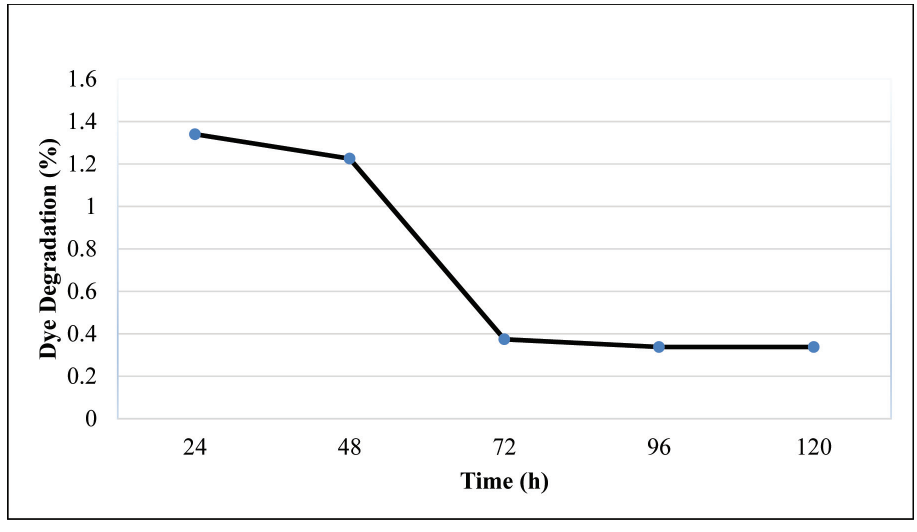

Figure 3: Decolourization of 40% 3-Nitro-1,5-di-m-tolylformazan

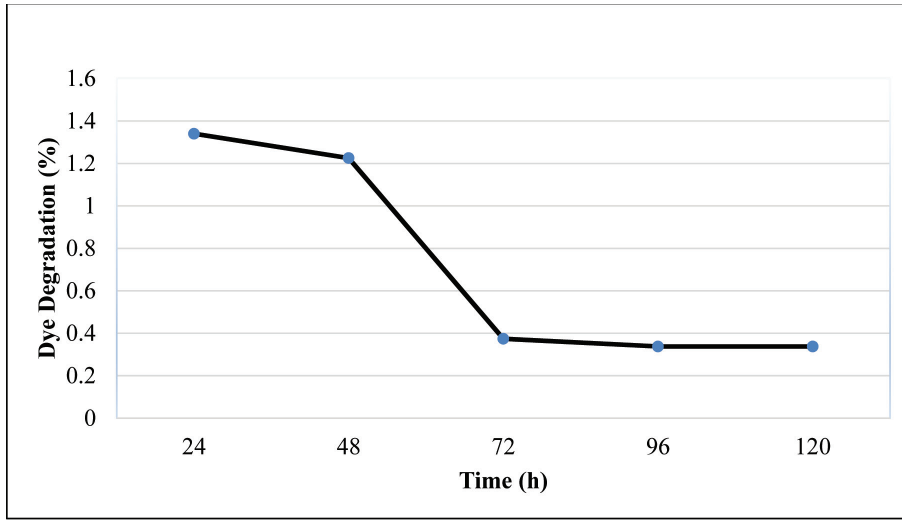

Figure 4: Decolourization of 60% 3-Nitro-1, 5-di-m-tolylformazan

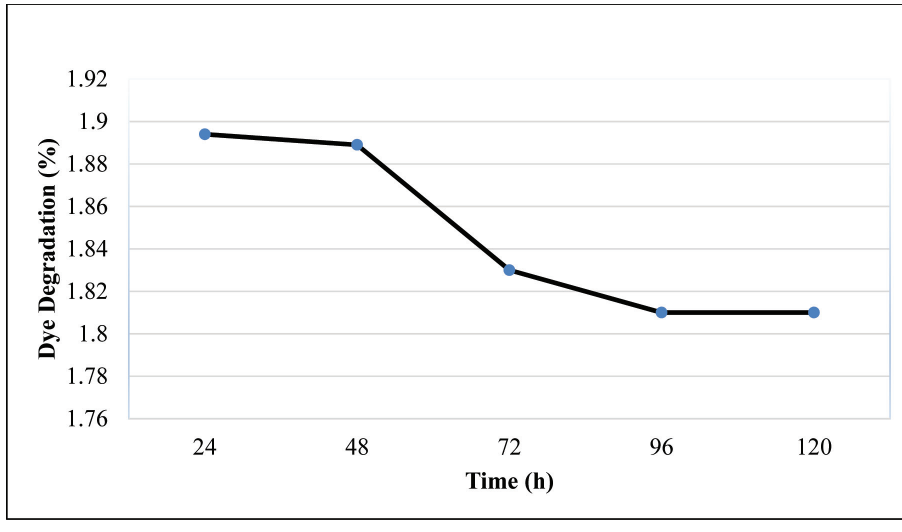

Figure 5: Decolourization of 80% 3-Nitro-1,5-di-m-tolylformazan

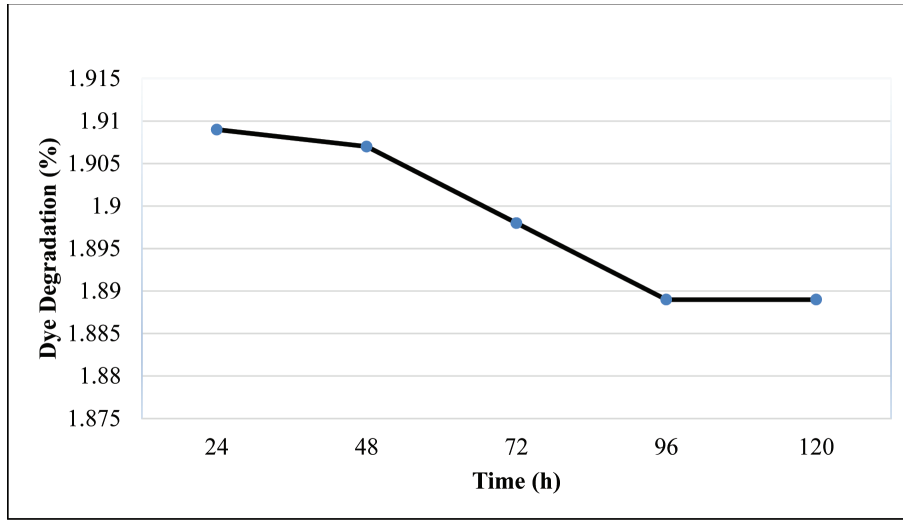

Figure 6: Decolourization of 100% 3-Nitro-1,5-di-m-tolylformazan

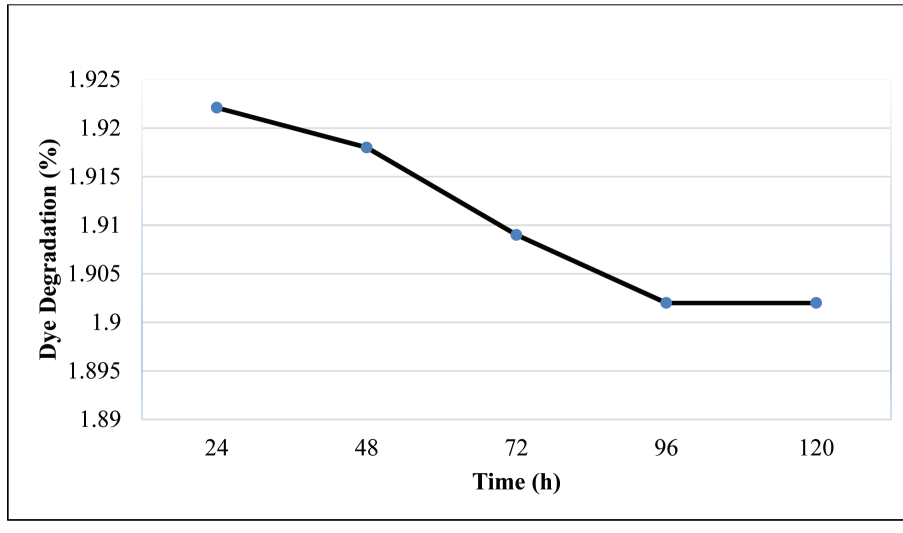

Figure 7: Decolourization of 20% 3-nitro-1,5-di-m-tolyl formazan by Coriolus versicolor

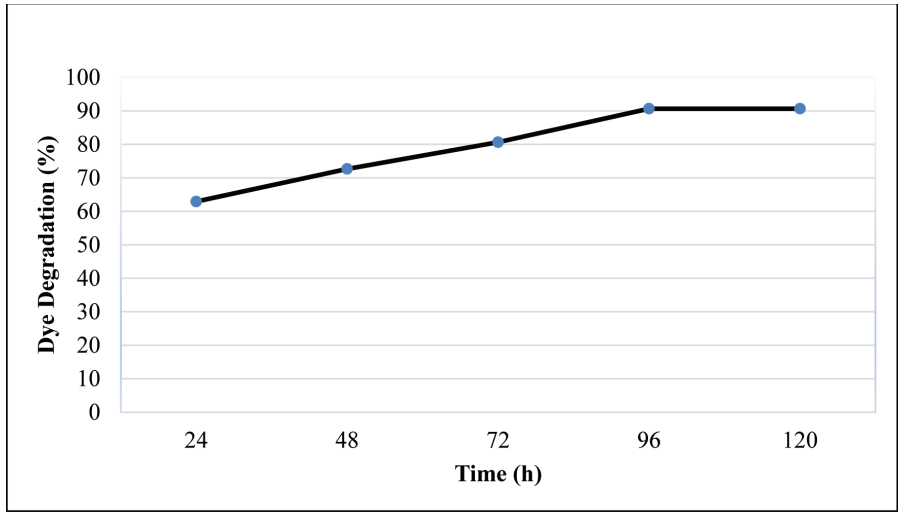

Figure 8: Decolourization of 40% 3-nitro-1,5-di-m-tolylformazan by *Coriolus versicolor*

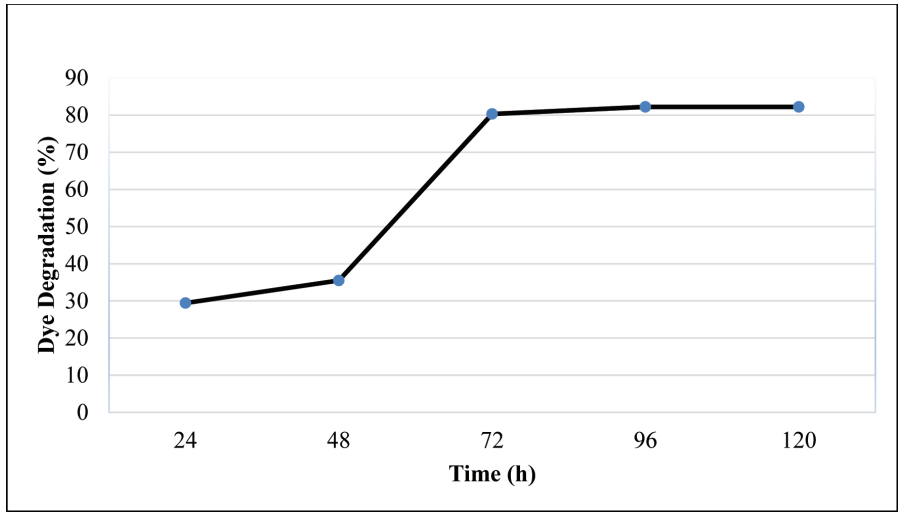

Figure 9: Decolourization of 60% 3-nitro-1,5-di-m-tolylformazan by *Coriolus versicolor*

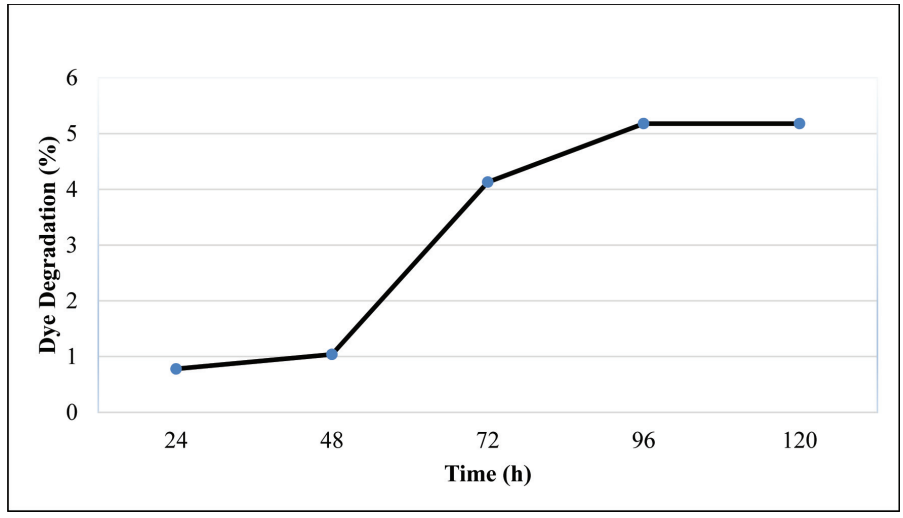

Figure 10: Decolourization of 80% 3-nitro-1,5-di-m-tolylformazan by *Coriolus versicolor*

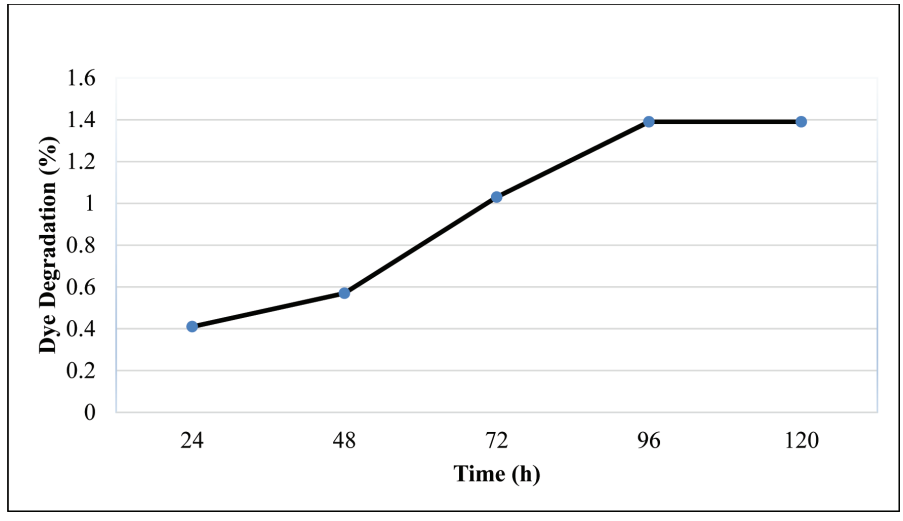

Figure 11: Decolourization of 100% 3-nitro-1,5-di-m-tolylformazan by *Coriolus versicolor*

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