GLOBAL JOURNAL OF BIO-SCIENCE AND BIOTECHNOLOGY

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USE OF FUNGI ISOLATED FROM TEXTILE EFFLUENT FOR DEGRADATION OF SYNTHETIC DYES AND OPTIMIZATION OF DEGRADATION PROCESS

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ABSTRACT
In the present study, for isolation of harmful synthetic dye degrading microorganisms (fungi), samples were collected from different forest regions (near textile industries), soil samples near from textile and dye industries waste were also used. Out of different fungal isolate, one potent dye degrading fungus namely *M.G.T 1* was selected for further study, using slide culture and microscopic observation, it was found that fungal strain *M.G.T 1* is *Aspergillus Spp.* Dye decolourization efficiency of selected fungi was determined. Initially the activities against 08 different synthetic dyes were determined. Four dyes namely Thymol blue, Malachite green, Bromophenol blue and Bromocresol purple shows notable % of degradation. Decolourization efficiency of free fungal mycelium was determined and it was found that maximum % degradation was obtained against Bromocresol purple (89.63%). For the increasing % degradation value physico-chemical parameter were optimized and it was found that isolated fungal *spp*. can give best % degradation value for Bromocresol purple when 1.0 ml of inoculum was added in the dye solution of pH 7 with agitation at 80rpm and incubation for 120 hrs.

KEYWORDS: Dye Degradation, Fungi, Textile effluent, Synthetic dyes.

INTRODUCTION

Environmental pollution from human activities is a major challenge for the present world. Textile, cosmetics, pharmaceuticals and dying industry effluents constitute a major source of water pollution. The waste water generated from textile industries vary in their characteristics depends on the process employed such as desiring, scouring, bleaching, mercerizing, dyeing, printing and finishing. The concentration of dye contained in the effluent varies between 10-200 mg/ml depending on the dyeing process and type of treatment method employed. Many dyes and pigments are hazardous and toxic for human as well as for aquatic life in the concentration at which they are being discharged to receiving water bodies (Rohilla *et al*., 2012). Industrial dyes can be released into the environment from two major sources: as effluents from synthesis plants and from dye-using industries, such as textile factories. It is estimated that between 10 and 15% of the total dye used in the dyeing process may be found in waste water. Several of these dyes are very stable to light, temperature, and microbial attack, making them recalcitrant compounds. About 50% of the industrial colorants produced in the world are azo dyes. These can be transformed to carcinogenic compounds under anaerobic conditions. Ligninolytic fungi have been reported to degrade xenobiotic compounds. The enzymatic systems that involve the enzymes of lignin degradation are able to transform polycyclic aromatic hydrocarbons, chlorinated phenols, PCB, dioxins, pesticides, explosives, dichloroaniline, and dyes. The white rot fungus *Phanerochaete chrysosporium* is able to decolorize several industrial dyes and polymeric dyes (Rodriguez *et al*., 1999). Azo dyes, which represent about one-half of all dyes in common use, are employed as coloring agents in the food, pharmaceutical, and textile industries. The popularity and widespread use of azo dyes is due to several factors. As a group, they are colour-fast and encompass the entire visible spectrum, and many are easily synthesized from inexpensive and easily obtained starting materials (Zhao, 2004). It is important to consider the optimization of a medium such that it meets as many as possible of all the criteria given. The meaning of optimization in this context does need careful consideration (Winkler, 1991). When considering the biomass growth phase in isolation it must be recognized that efficiently grown biomass produced by an 'optimized' high productivity growth phase is not necessarily best suited for its ultimate purpose, such as synthesizing the desired product. Different combinations and sequences of process conditions need to be investigated to determine the growth conditions which produce the biomass with the physiological state best constituted for product formation. There may be a sequence of phases each with a specific set of optimal conditions. (Stanbury *et al*., 1995). New processes for dye degradation and wastewater treatment and reutilization are being developed, (Santos *et al.*, 2007). In particular, systems based on biological processes using a large variety of bacterial strains, allow for degradation and mineralization with a low environmental impact and without the use of potentially toxic chemical substances, under mild pH and temperature conditions. These microorganisms have the ability not only to decolorize dyes but also to detoxify it (Ndas *et al*., 2011).In the present study one potent dye degrading fungus was used to study the dye decolourization efficiency. Initially the

activities against 08 different synthetic dyes were determined. Maximum % decolourization was shown by four dyes namely Thymol Blue, Malachite Green, Bromophenol Blue and Bromocresol Purple. Different parameters for degradation of Bromocresol Purple were also optimized.

MATERIALS & METHODS

Isolation, Screening & Characterization of Potential Strain

Various textile effluent and soil samples were collected for isolation of potential dye degrading microorganisms (fungi). Sampling has been carried out randomly using sterile plastic bags and sterile bottles. Two cycles of enrichment have been carried out by successive transfer of pre-enriched samples into fresh media. The samples from last enrichment were used for isolation.

The isolated fungi were further subjected to screening. The organism which shows good % degradation activity was selected for further study. The selected fungal strain was identified by using morphological analysis of fungal hypal morphology by using microscopic observation.

Dye degradation study

Dye decolourization efficiency of isolated fungus was determined. Initially the activities of fungi against 08 different selected synthetic dyes were determined by using 100ppm concentration of each dye. Table no.1 shows list of 08 selected dyes for decolourization experiments. The lambda Max value of each dye was obtained by measuring the absorption of each dye at various wave-lengths.

TABLE 1: List of selected dyes with molecular formula and molecular weight

Sr.No	Dye Name	Molecular Formula	Molecular Mass
1	New Fuchsin	$C_{22}H_{23}N_3.HCI$	365.91 g/mol^{-1}
2	Fuchsin Acid	$C_{20}H_{17}N_3Na_2O_9S_3$	585.538 g/mol^{-1}
3	Thymol Blue	$C_{27}H_{30}O_5S$	466.59 g/mol^{-1}
4	Crystal Violet	$C_{25}N_3H_{10}CI$	407.979 g/mol^{-1}
5	Malachite Green	$C_{23}H_{25}CIN_2$	364.911 g/mol^{-1}
6	Neutral Red	$C_{15}H_{17}CIN_4$	288.78 g/mol^{-1}
7	Bromocresol Purple	$C_{21}H_{16}Br_2O_5S$	540.22 g/mol^{-1}
8	Bromophenol Blue	$C_{19}H_{10}Br_4O_5S$	669.96 g/mol^{-1}

CALCULATION

For calculating % decolourization of dye following formula was used

% Decolourization = (Initial absorbance - Final absorbance)/ (Initial absorbance) x 100

For the preparation of stock solution 0.1 gm of each dye powder is dissolved in 10 ml of suitable solvent which gives final concentration of 1% (10 mg/ml).

The desired dye concentration (100ppm) was prepared from the stock solution in 250 ml conical flask containg 100 ml MSM medium. Then equal amount of fungal Inoculum is added in each flask. Incubation is carried out in 37^0C at 120rpm. Decolourization activity was determined by monitoring the decrease in absorbance on a spectrophotometer at lambda max of each dye.

By measuring the initial and final absorbance, percent dye decolourization was calculated. One dye (Bromocresol purple) is selected for further optimization studies on the basis of result obtained.

Optimization of parameter

For the development of inoculum, 100ml of MSM media containing desire concentration (100ppm) of Bromocresol purple was inoculated with 5ml inoculums containing 7days old culture of fungi & incubated at 25°C at 150 rpm for growth and degradation of dyes. The selected process parameters were optimized *viz*. Inoculum level, Agitation, Time of incubation and pH.

Effect of inoculum level on dye degradation

The effect of different concentration of seven days old inoculum (0.2, 0.4, 0.6, 0.8, 1.0 and 1.2 ml) of *Aspergillus* on degradation efficiency were determined by inoculating in the MSM medium containing dye solution of selected concentration (100ppm). After incubation for 24hrs. Sample were removed and subjected to spectroscopic analysis.

Effect of agitation on dye degradation

The effect of agitation on dye degradation was determined by incubating MSM broth containing fungi at various rpm (Revolution per minute) like 20,40,60,80,100 for 24 hrs. and degradation activity was studied by recording the initial and final absorbance of dye.

Effect of time of incubation on dye degradation

The MSM medium was used for determination of optimum time of incubation for dye decolourization. The samples were withdrawn after every 24 hrs. and analyzed by spectrophotometric analysis.

Effect of pH on dye degradation

The MSM medium of different pH (5 to 9) was used for determination of optimum pH. The influence of pH on dye degradation activity was studied by recording the initial and final absorbance of dye after incubated at 25ºC for 24 hrs. and absorbance were recorded at lambda max of selected dye.

RESULTS & DISCUSSION

Isolation, screening and characterization of potential strain

From the enriched culture broth 10 fungal isolate were obtained which shows good dye removing ability were selected and subjected for screening procedure.

The 10 positive fungal isolate were screened on the basis of % dye degradation activity. It is observed that 1 presume to be potent, 7 showed medium potency and 2 showed weak activities. The organism which showed rapid growth and best degradation ability (*M.G.T 1*) was selected for further study.

Microscopic study of the isolated fungal strain showed that it is having conidial heads, are short columnar and biseriate. Conidiophores are usually short, brownish and smooth walled, Conidia are globose and rough walled. On the basis of this observation it is concluded that the given fungal isolate *M.G.T 1* is *Aspergillus spp.*

Dye degradation study

The initial and final spectroscopic reading was used to calculate % decolourization which shows that isolated fungus show maximum dye degradation activity against Bromocresol purple (89.63%) followed by Thymol blue (84.39%), Malachite green (49.54%) and Bromophenol blue (49.11%)

It also gives good result against Phenol red (42.01%), New Fuchsin (36.91%) and Fuchsin acid (29.58%). The dye namely Neutral red and Crystal violets are not showing susceptibility to dye degrading enzyme (*e.g.* Laccase) produced by fungi as they give minimum percent degradation value 5.45% and 16.90% respectively. On the basis of % Decolourization activity Bromocresol purple were selected for further study.

TABLE 2: % Dye degradation activity of eight dyes

Sr. No	Dyes (100 ppm)	Initial	Final	% Decolourization
1	Thymol Blue	0.205	0.032	84.39%
2	Fuchsin Acid	1.335	0.940	29.58%
3	Crystal Violet	1.715	1.425	16.90%
4	New Fuchsin	2.121	1.338	36.91%
5	Malachite Green	1.439	0.726	49.54%
6	Neutral Red	0.055	0.052	5.45%
7	Bromocresol Purple	1.167	0.121	89.63%
8	Bromophenol Blue	2.834	1.442	49.11%

OPTIMIZATION OF PARAMETER

Effect of inoculum concentration on dye degradation

The Influence of the volume of Inoculum on decolourization of the dye Bromocresol Purple solution by *Aspergillus spp.* is presented in (Fig. 1) From the data it is observed that optimum concentration of Inoculum of fungi effective for decolorizing Bromocresol Purple is 1.0ml (65.85%). A very low concentration of Inoculum (0.2 or 0.4) leads to increase the time require for development of biomass which adversely affect the percent decolourization value as compare to high concentration of the same (0.8 or 1.0). Further increase in Inoculum concentration above 1.0 ml did not show significant rise in degradation activity.

Kumar Praveen G. N. and Sumangala K. Bhat (2012) found ideal volume of inoculum was 2% for *P. chrysogenum* and 10% for *A. niger*. While we found that *A. nidulans* gives comparatively good result at 1% Inoculum level.

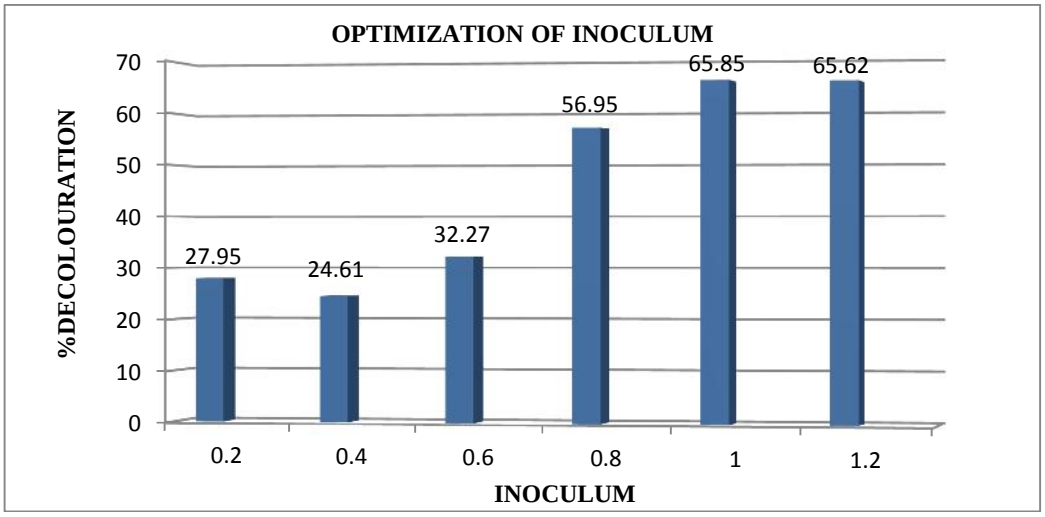

FIGURE 1: Effect of Inoculum concentration on dye degradation

Effect of agitation on dye degradation

The effect of agitation on dye degradation was determine and it was found that fungus *Aspergillus* shows linear increase in percent degradation activity with increasing rpm from 20 to 80. Further increase in rpm shows negative effect on colour removal ability of fungus. The result is in agreement with the result obtained by other author (V. Gopi *et al*) which suggest that constant shaking (Agitation) is important parameter which is to be monitored carefully during the dye degradation processes. Fungal mycellial growth, development as well as enzyme production is depend on aeration and nutrients availability which can be achieved by providing optimum agitation condition.

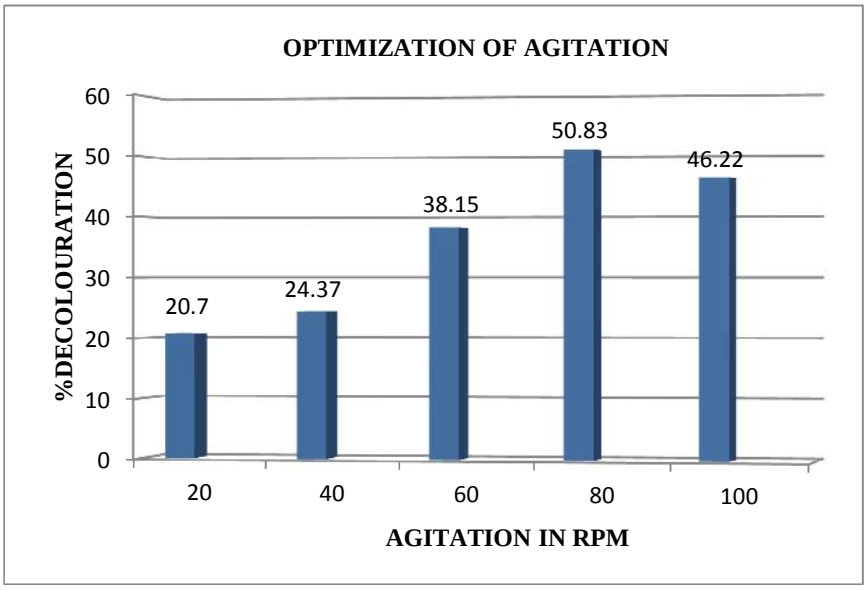

FIGURE 2: Effect of Agitation on dye degradation

Effect of time of incubation on dye degradation

The effect of time course on decolourization of Bromocresol Purple under optimum conditions by *Aspergillus spp.* is illustrated in (Fig. 3). The results indicate the fact that fungus capable of removing nearly 65.81% of dye colour when incubated for 120 hrs. on rotary shaker (80 rpm) at 37^0C. Further increase in time of incubation did not show any significant effect on degradation activity. This is may be due to depletion of nutrient from the medium and accumulation of some toxic secondary metabolite which inhibit fungal growth and show negative effect on overall dye degradation activity. Similar kind of result was obtained by V. Gopi *et al.*

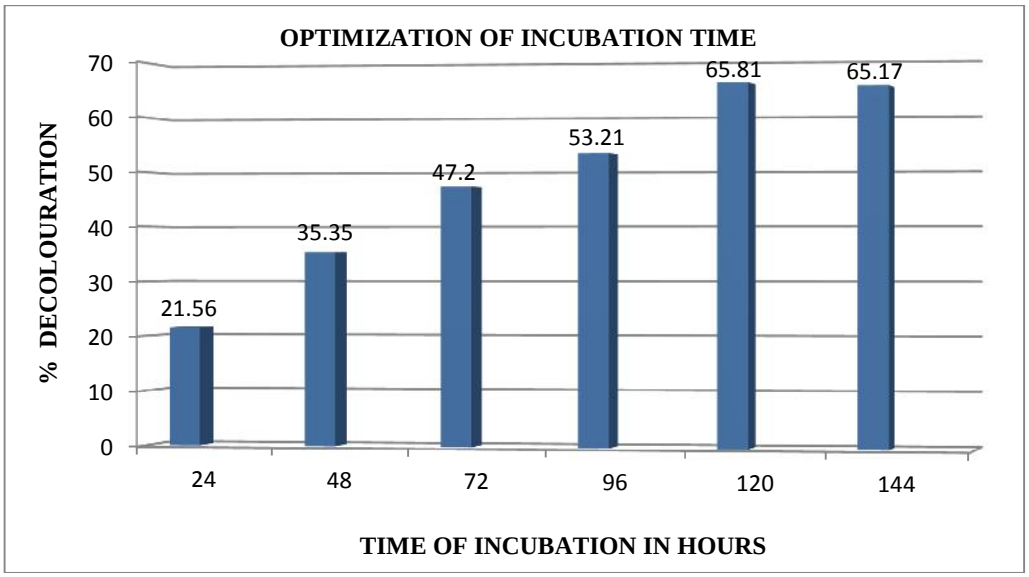

FIGURE 3: Effect of Time of Incubation on dye degradation

Effect of pH on dye degradation activity

(Fig. 4) illustrates the effect of different pH on decolourization of Bromocresol Purple by *Aspergillus* from the data it can be concluded that fungi is more efficient in decolorizing Bromocresol Purple at pH 7 (71.22%) which is ideal for its activity under in vitro condition. More acidic or more alkaline condition is not suitable for number of fungal enzymes (*e.g.* Laccase) which play major role in synthetic dye degradation pathways. So dye degradation activity of fungi decrease with increasing or decreasing medium pH. And it is maximum at neutral (7) pH. This result match with the result obtained by Kumar Praveen G.N., Sumangala K. Bhat they found maximum activity of *P. chrysogenum* in the range of pH 7 to 8, they also observe maximum decolourization activity of *Cladosporium sp.* at pH 6.

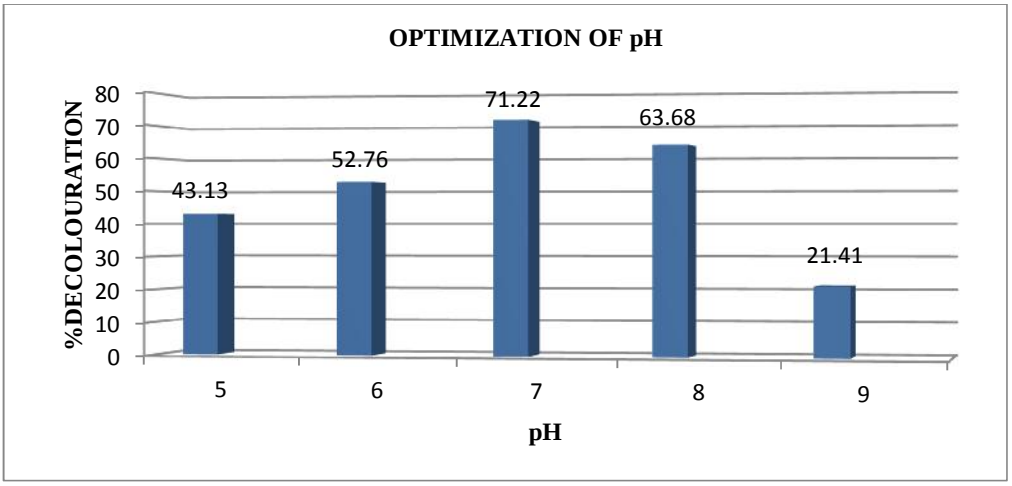

FIGURE 4: Effect of pH on dye degradation

CONCLUSION

Dye decolourization efficiency of *Aspergillus spp.* was determined. Initially the activities against 08 different synthetic dyes were determined. Out of the selected dyes, Bromocresol Purple shows best result. The effect of four parameter on dye degradation process was determine and it was found that isolate show best % degradation of Bromocresol Purple when 1.0 ml of Inoculum was added in the dye solution of pH 7 with agitation at 80rpm and incubation for 120 hrs.

So the present study conclude that fungi can serve as one of the environment friendly and coast effective option for removing harmful colour from the contaminated water of industries and more research should be carried out in this area for transferring it on large scale.

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