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Research Article

Biodegradation of synthetic textile dyes by thermophilic lignolytic fungal isolates

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Abstract: Synthetic dyes are extensively used in different industries like textile dyeing, paper, printing, color, photography, pharmaceutics and cosmetics. These are generally toxic and carcinogenic in nature. If not treated, they will remain in nature for a long period of time as they are recalcitrant. Among these, azo dyes represent the largest and most versatile class of synthetic dyes. Approximately 10-15% of the dyes are released into the environment during manufacture and usage. Various methods are used for dye removal viz. physical, chemical, electrochemical and biological. Advantage of chemical, electrochemical and biological methods over physical involves the complete destruction of the dye, but chemical and electrochemical methods are found to be expensive and have operational problems. So the biological method is preferred over other methods for degradation/decolorization of dyes. In the present study, thermophilic lignolytic fungal culture was isolated from compost/soil/digested slurry/plant debris, were subjected for acclimatization to Remazol Brilliant Blue (RBB) at 0.05% concentration, in the malt extract broth (MEB). The most promising fungal isolates were used for further dye degradation studies. The results suggest that the isolates T10, T14 and T17 as a useful tool for degradation of reactive dyes.

Keywords: Biodegradation, Remazol Brilliant Blue, Synthetic dyes, Thermophilic fungi.

1. Introduction

The reactive dyes are used extensively in textile industries due to their favorable characteristics of bright color, water-fast and simple application techniques with low energy consumption [1]. Many contaminants are present in wastewater, such as acids, bases, toxic organic and inorganic dissolved solids, and colors. Among them, color and causative compounds are always undesirable [2]. It affects the water quality and may become a threat to public health, as they are highly toxic and carcinogenic. The effluents with high levels of biological oxygen demand (BOD) and chemical oxygen demand (COD) values are highly toxic to biological life [3]. There are over 10,000 commercially available dyes with a production of over 7×10^5 tons per year. Azo dyes account for almost 60 to 70% of all the synthetic dyes produced globally [4]. Dyes are made up abundant class of organic compounds characterized by the presence of unsaturated groups (chromophores) such as –C=C-, -N=N- and –C=N-, which are responsible for their coloration and when such a bond is broken the compound loses its color. Out of them Azo dye is the largest and most versatile class of dye but has structural properties that are not easily degradable under natural conditions. Discharging of such complex molecules into receiving streams not only affects the aesthetic aspects but also interferes with transmission of sunlight into streams and therefore reduces photosynthetic activity [5]. Without adequate treatment, such dyes will remain in the environment for an extended period of time [6].

Various physicochemical methods, such as adsorption on activated carbon, electrocoagulation, flocculation, ion exchange, membrane filtration, ozonation, and reverse osmosis have been used for degradation/decolorization of dyes in wastewater. However, these methods are less efficient, more costly, of limited applicability, and produce wastes, which are difficult to dispose of [7]. The degradation of azo dyes produces aromatic amines, which are carcinogenic and mutagenic. Recently, several reports appeared showing

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that the microorganism has the ability not only to decolorize dyes but also to detoxify it. Compared with chemical/physical methods, microbial processes have received more interest because of their cost-effectiveness, lower sludge production and environmental-friendliness [1]. The most widely studied class of microorganisms in regard to dye degradation and decolorization are the bacteria. Several isolates of *Pseudomonas*, *Alcaligenes*, *Acinetobacter* and *Bacillus* are important bacteria useful in bioremediation of halogenated organic compounds [8]. The most widely researched fungi in regard to dye degradation are the lignolytic fungi [9]. These have received extensive attention due to powerful production of lignolytic enzymes and their unique ability to degrade lignin to CO_2 [10]. Such an extent of degradation is due to the strong oxidative activity and lower substrate specificity of their lignolytic enzyme system, composed of laccase, lignin peroxidase and manganese peroxidase. Reports were available of degradation or decolorization of reactive blue dye by mesophilic fungi [11], but there were limited data available on decolorization of reactive dyes by thermophilic fungi. Therefore, the possibility of bio-decolorization of RBB was exploited with the objectives of the screening of thermophilic cultures capable of decolorizing the dye and determination of percent degradation.

2. Material and Methods

Screening of isolated cultures for dye degradation/decolorization: The thermophilic lignolytic fungi were selected from previous study [12]. These selected isolates were screened for their dye degradation/decolorization potential. In order to examine the effect of cultures for the dye decolorization, 0.05% of RBB was added to the malt extract broth (MEB: 2% malt extract) in 250ml flask and autoclaved at 121°C at 15psi for 15 min. After cooling the flasks were inoculated with 1-2 bits of fungal isolates and incubated at 50°C temperature. Decolorization was ranked visually (intense 4; good 3; moderate 2; slight 1; no decolorization).

2.1 Dyestuff and chemicals

All chemicals used were of the highest purity and of analytical grade. Malt Extract Broth (MEB) and Remazol Brilliant Blue 250 (RBB) were purchased from Hi-Media, Mumbai, India.

2.2 Selection of model dye

The wide range of reactive dyes was used for the degradation or decolorization studies. Remazol Brilliant Blue, RBB (empirical formula:- $C_{27}H_{23}N_5Na_4O_{20}S_6$), an anthraquinone dye, one of reactive blue dye, selected as a model dye because it is structurally rigid and extensively used in textile industries (Fig. 1).

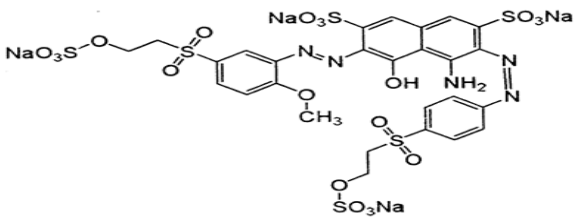

Fig. 1. Structure of Reactive Blue 250.

2.3 Determination of optical density (OD)

The percent decolorization was measured at regular interval of 48 hr and the optical density was measured at 610nm [11]. Abiotic control (without fungi) was always included. The % of decolorization was measured as per following formula:

$$\% \text{ Decolorization} = \frac{\text{Initial absorbance} - \text{final absorbance}}{\text{Initial absorbance}} X100$$

3. Results and Discussion

3.1 Screening of fungal cultures

These isolates were screened for their dye degradation/decolorization potential and were ranked on the basis of visual identification (Table 1). It can be seen that isolate no. T10, T14 and T17 showed maximum hydrolysis followed by T5 and T7. The isolate T10 decolorized the dye substrates to a great extent up to the 10th day while isolate no. T47 gave negative results. So T10, T14 and T17 were selected for quantitative studies.

Table 1. Ranking of RBB decolorizers/degraders based on visual identification.

S. No.	Isolate No.	Ranking of decolorization/degradation
1	T1	2
2	T4	2
3	T5	3
4	T7	3
5	T10	4
6	T12	2
7	T14	4
8	T17	4
9	T22	1
10	T25	2
11	T32	2
12	T35	1
13	T42	2
14	T44	2
15	T47	1
16	T56	1
17	T65	2
18	T72	2
19	T74	1
20	T77	2

Key= 4: intense hydrolysis, 3: good hydrolysis, 2: moderate hydrolysis, 1: slight hydrolysis, -: no hydrolysis

3.2 Dye degradation/decolorization Studies in broth media

Results from Table 2 showed optical density (610nm) and % dye degradation/decolorization of

selected isolates. Isolate no. T10 showed maximum percent dye decolorization of 42.98%, followed by T17 which showed 41.64% dye decolorization at the 10th day of incubation at a concentration of 0.05% dye. There was neither growth nor decolorization in the control. This showed that decolorization was due to metabolic activity of the organisms. Decolorization of synthetic dyes is the result of the cleavage of the chromophoric group which generates colorless metabolic intermediates. The intermediate metabolite of the dye substrate is aromatic amines. The cleavage of the chromophoric group of dyes is a reduction process which requires redox equivalents (electron donors) that transfer electrons to the chromophoric group (electron acceptor) of dye [13, 14]. Ring opening of the aromatic moiety of the dyes were the sole source of carbon and energy. Utilization of azo-nitrogen of dye provided the nitrogen requirements of the organism. Aromatic amines generated by the reductive cleavage of Remazol brilliant blue and the parent molecules are potentially toxic, mutagenic and carcinogenic [15, 16]. The utilization of these dyes as sole sources of carbon and nitrogen may detoxify the parent compounds or their metabolic intermediates.

Table 2. Percent dye degradation/decolorization of selected isolates.

Isolate No.	Incubation time (days)	Absorbance (610 nm)	% dye degradation/ Decolorization
Control	0	2.980	0
T 10	2	2.662	10.67
	4	2.506	15.91
	6	2.310	22.48
	8	2.186	26.64
	10	1.699	42.98
T14	2	2.899	2.72
	4	2.856	4.16
	6	2.525	15.27
	8	2.188	26.58
	10	1.996	33.02
T17	2	2.848	4.43
	4	2.801	6.01
	6	2.702	9.33
	8	2.113	29.09
	10	1.739	41.64

4. Conclusion

Based on the above study, it may conclude that fungal isolates possess potential to degrade and decolorize synthetic dyes and more work is needed for exploring novel strains or consortia.

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