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UV Induced Mutagenesis In Bacteria And Fungi: An Attempt To Increase Dye Decolourisation Efficiency.

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ABSTRACT

A dye is used to give color to materials and is extensively used in the textile, leather, pharmaceutical and cosmetics industries, pose a threat for all life forms. The physico-chemical method of industrial effluent treatment does not remove the dyes effectively. Microbial method of dye decolorization is eco-friendly in nature. Therefore aim of the present work was to study the effect of UV rays on some dye decolourising fungi and bacteria by using malachite green and methylene blue. The decolorization efficiency of the *Bacillus subtilis* NCIM 2063 was studied for ultraviolet radiation exposure for 3, 6, 9 and 12 minutes. The results obtained showed that there was no increase in decolourisation percentage with the increase in exposure time period. On the other hand, decolourisation efficiency of *Aspergillus niger* NCIM 548 increased with increase in UV exposure time period up to 9 minutes and after that it decreases which indicated that UV induced mutagenesis may play important role in strain improvement.

Keywords: Dye Decolourisation, *Bacillus subtilis, Aspergillus niger*

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INTRODUCTION

The growth of the world population, modernization of industries and modernization of agriculture has overloaded water, atmosphere and soil with pollutants. The textile industries consumes large amount of water and thus produces large amount of wastewater comprising dye. Therefore, it has to be really concerned in releasing these types of wastewater to the environment. In the disposal of textile wastewater, colour is very important due to the aesthetic deterioration as well as the obstruction of penetration of dissolved oxygen and sunlight into natural water bodies. Discharge of polluting wastewater from industrial sources is a real problem in several countries. This situation is even worse in developing countries like India where little or no treatment is carried out before the discharge [1].

Most widely used dyes in market are azo dyes and comprise a diverse group of synthetic chemicals that are widely used by the textile, leather, food, cosmetics, and paper product industries. Approximately 1 million tons of azo dyes are produced worldwide each year [2]. It is estimated that about 2% of these dyes are discharged in aqueous effluents during the manufacturing process [3]. Treatment of dye containing textile industrial effluents by commonly used physical and chemical methods is difficult because of their high biological oxygen demand, chemical oxygen demand, heat, colour, pH and the presence of metal ions [4].

Much research has been performed to develop effective treatment of dye containing wastewater [5]. 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 [6] [7]. Fungi have the ability to convert wide variety of hazardous chemicals into nonhazardous one. Due to this ability fungi are increasingly used in bioremediation [8].

The use of microorganisms for the treatment of dye containing industrial effluents offers considerable advantage because of its low cost and simple method [9]. Most important advantage of using microorganisms for the treatment of dye containing wastewater is that the end products of complete mineralization are not toxic [10]. Their enzyme producing activity makes them effective decolourizers. They remove toxic metals by bioadsorption ultimately rendering the effluents more ecofriendly [11].

Ultraviolet light is electromagnetic radiation ranging from 100 to 300 nm in wavelength. High dosages of UV radiation causes DNA damage which further leads to cell death. UV light has poor penetration power. It cannot pass through solid objects, including glass and plastic, thus, it is commonly used for killing microorganisms on nonporous surfaces and only under conditions where direct exposure to the eyes or skin is not possible [12]. The objective of the present research work was to study the effect of UV rays on some dye decolourising fungi and bacteria.

MATERIALS AND METHODS

Collection of Microorganisms: *Bacillus subtilis* NCIM 2063 and *Aspergillus niger* NCIM 548 were procured from National Chemical Laboratory (NCL), Pune. The cultures were maintained on nutrient agar and Saboraud's Dextrose agar slants respectively.

UV Treatment:-

For bacteria: *Bacillus subtilis* was streaked on five plates of nutrient agar labeled as A, B, C, D, E. The plates were exposed to UV radiation for 0, 3, 6, 9, 12 minutes respectively. After UV treatment the plates were incubated at 37^0C for 24 hours.

For fungi: *Aspergillus niger* was inoculated on five plates of Sabourauds Dextrose agar labeled as A, B, C, D, E. The plates were incubated at 28^0C overnight for generation of spores. The plates labeled as A, B, C, D, E were exposed to UV radiation for 0, 3, 6, 9, 12 minutes respectively. After UV treatment the plates were incubated at 28^0C for 7-15 days [13].

Assay of Decolourisation Activity:-

For Bacteria and Fungi:

Malachite green and Methylene blue were obtained from Hi-Media Laboratories Pvt. Ltd., Mumbai. Dye decolourisation experiments were carried out in five, 250ml conical flasks labeled as A, B, C, D, E each for malachite green and methylene blue. In five flasks each 100ml nutrient broth for bacteria and in another five flasks each 100ml Saborauds Dextrose broth for fungi was added. It was carried out for both dyes malachite green and methylene blue. A 500µg/l dye concentration was added in each flask. All the flasks were then sterilized by autoclaving at 121^0C for 15 minutes. All the labeled flasks were inoculated with *Bacillus subtilis* and *Aspergillus niger* in respective media and exposed to UV radiation for 0, 3, 6, 9, 12 minutes respectively for each dye. All the inoculated flasks of bacteria were kept in shaker incubator at 37^0C for 48 hours and at 130 rpm while that of fungi were kept in shaker incubator at 28^oC for 5 days at 130 rpm. After incubation 2ml of sample from bacterial inoculums was taken and centrifuged at 16000 rpm for 20 minutes in order to prevent absorbance interference from cellular or suspended particles [14]. However, after incubation 10 ml of the sample from fungal inoculums was filtered by whatman filter paper and centrifuged at 6000rpm for 10 minutes [15].

Decolourisation of the dyes could be seen visually and determined by measuring optical density of supernatant at respective absorbance maxima. Uninoculated flask served as control. Decolourisation efficiency was expressed according to the following formula-

$$\text{Decolorization (\%)} = \frac{\text{Initial absorbance} - \text{Observed absorbance}}{\text{Initial absorbance}} \times 100$$

RESULTS AND DISCUSSION

Among various industries, the textile dying industries discharge large volume of waste water after dyeing process [16]. Various contaminants present in industrial effluents like acids, bases, toxic organic and inorganic materials, dyes etc. causes water pollution Dyes are considered among most harmful contaminants because of their complex aromatic molecular structure which makes them difficult to biodegrade. Various physical and chemical methods used for treating textile effluents are less efficient and releases substances which are not eco- friendly. Bioremediation using microorganisms like bacteria and fungi is much efficient and eco-friendly technique of treating textile effluents. The present study was aimed to study the effect of ultraviolet radiations on dye degrading fungi and bacteria.

Dye Decolourisation Assay for Bacteria *Bacillus subtilis*:-

In nutrient broth decolourisation of malachite green and methylene blue was studied by *Bacillus subtilis* NCIM 2063. In nutrient broth decolourisation of malachite green was found to be 100% while that of methylene blue was 45% by *Bacillus subtilis* in 48 hrs.

Effect of UV Radiations on Dye Decolourisation by Bacteria *Bacillus subtilis*:-

The decolorization efficiency of the *Bacillus subtilis* NCIM 2063 exposed to ultraviolet radiation for 3, 6, 9, 12 minutes, then decolorization of malachite green and methylene blue was studied. The results obtained showed that there was no increase in decolourisation percentage with the increase in exposure time period (Figure 1, 2). In present research it was found that in nutrient broth decolourisation of malachite green was 100% and of methylene was 45% by *Bacillus subtilis* NCIM 2063. The similar experiment was carried out in which it was revealed that decolourisation of malachite green was 100% by isolated bacterium species *Bacillus strain* MTCC 3330 [14].

Dye Decolourisation Assay for Fungi *Aspergillus niger*:-

In sabouraud dextrose broth decolourisation of malachite green and methylene blue was studied by the *Aspergillus niger* NCIM 548. In the present investigation it was found that in sabouraud dextrose broth, decolourisation of malachite green was 64% and methylene blue was 40% by *Aspergillus niger* NCIM 548.

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These results were correlated with the previous findings [15] [17]. They reported the potential of dye degrading fungal species from textile mill effluents in ecofriendly way. They carried out the decolourising assay in liquid medium for malachite green and methylene blue. They showed that in liquid medium decolourisation of malachite green was 55% and methylene blue was 84.20% by *Aspergillus niger*.

Figure 1: Decolourisation of malachite green by *Bacillus subtilis* exposed to UV radiations for 0, 3, 6, 9, 12 minutes respectively.

Figure 2: Decolourisation of methylene blue by *Bacillus subtilis* exposed to UV radiations for 0, 3, 6, 9, 12 minutes respectively

Figure 3: Decolourisation of malachite green by *Aspergillus niger* exposed to UV radiations for 0, 3, 6, 9, 12 minutes respectively.

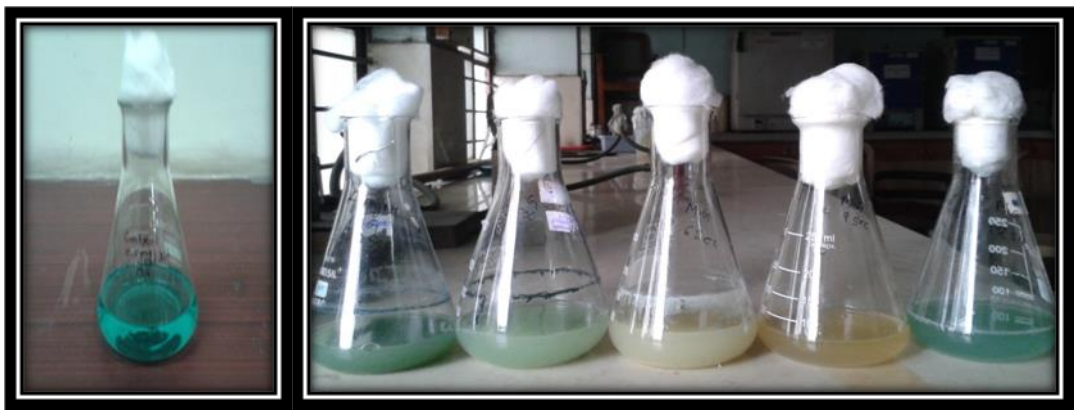

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Figure 4: Decolourisation of methylene blue by *Aspergillus niger* exposed to UV radiations for 0, 3, 6, 9, 12 minutes respectively

Effect of UV on Dye Decolourisation by Fungi *Aspergillus niger* :-

Aspergillus niger NCIM 548 exposed to ultraviolet radiation for 3, 6, 9, 12 minutes, then decolorization of malachite green and methylene blue was studied (Table 1) (Graph 1). For *Aspergillus niger* 60% to 93% of decolorization was observed in malachite green and 40% to 60% of decolorization was observed in methylene blue when it was exposed to UV radiation for different time interval (0, 3, 6, 9, 12 minutes). When *Aspergillus niger* was exposed to 9 minutes UV radiation, maximum decolorization of malachite green and methylene blue was observed. Maximum decolorization for malachite green was 93% and for methylene blue it was 60% relating to the exposure time period (Figure 3, 4). Results showed that for *Aspergillus niger* 64%, 70%, 90%, 93%, 63% of decolorization was observed in malachite green when exposed to 0, 3, 6, 9, 12 minutes ultraviolet radiation respectively. On the other hand 40%, 42%, 48%, 60%, 50% of decolourisation was observed in methylene blue by *Aspergillus niger* when exposed to 0, 3, 6, 9, 12 minutes ultraviolet radiation respectively.

Table 1: Effect of UV Radiations on Decolourisation by *Aspergillus niger*

Exposure to UV Radiations (minutes)	Decolourisation (%)	
	Malachite Green	Methylene Blue
0	64%	40%
3	70%	42%
6	90%	48%
9	93%	60%
12	63%	50%

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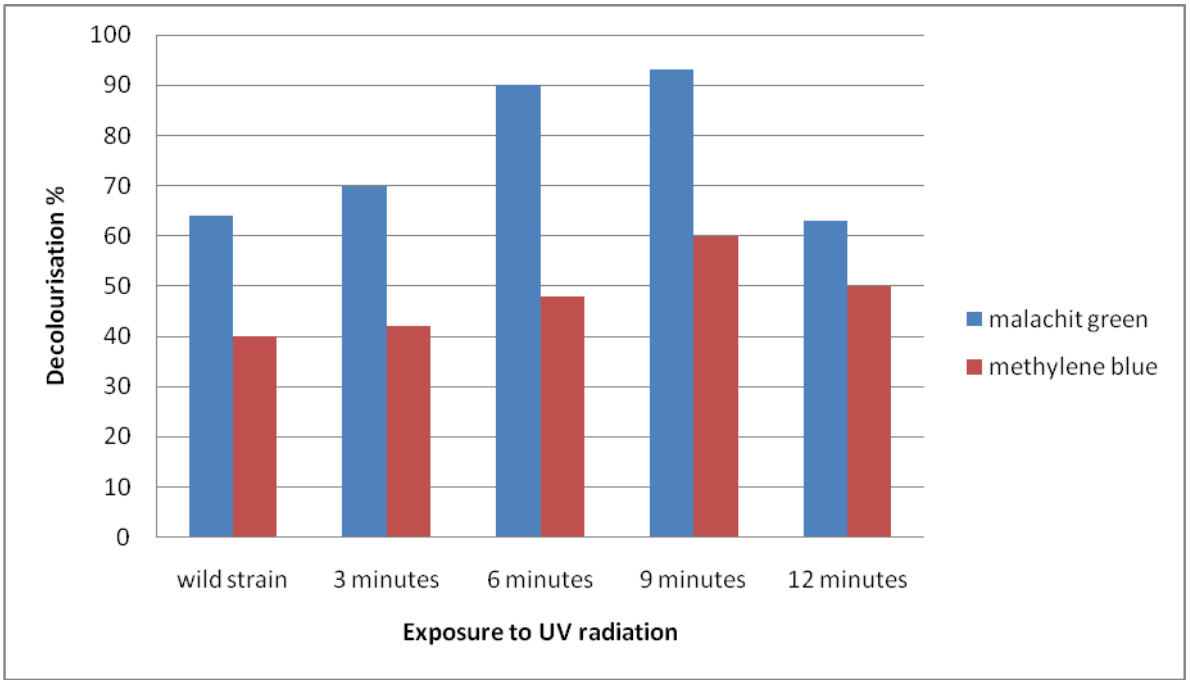

Graph 1: Effect of UV Radiations on Decolourisation by *Aspergillus niger*

In the previous study the wild fungal strain has been used and carried out a study using UV radiations. Their results showed that mutants that were exposed to 3,6,9,12,15 minutes to UV radiations showed 66%, 68%, 70%, 65%, 65% of decolourisation in malachite green respectively [13]. Decolourisation efficiency increased with increase in UV exposure time period up to 9 minutes and after that it decreases which indicated that UV induced mutagenesis may play important role in strain improvement thus supported present study.

CONCLUSION

Nowadays water pollution control is important in research. Various textile industries plays major role in water pollution by releasing toxic dyes. The physical and chemical methods used for treating water pollution are not ecofriendly and not economical. Present study showed that with the increase in UV radiations exposer time period of *Aspergillus niger,* the decolourisation percentage increased to some extent. But further increased in UV exposure time period there was decreased decolourisation percentage. Hence the present investigation concludes that exposure of the strain to UV radiation for short period of time leads to formation of mutants which increases percentage of decolourisation. Thus use of these UV radiation induced fungal mutants could be an environmentally friendly solution for treating textile dye pollution.

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