Marine soil isolated *Aspergillus flavus* assisted degradation of textile dyes

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Abstract

The main objective of this work is to develop cost-effective, simple and most effective approach for the degradation of textile dyes. In this concern, we have extracted the fungus from marine soil collected from Bay of Bengal, Visakhapatnam. Morphologically different colonies were collected and again streaked on potato dextrose agar plates by using streak plate method. Decolorization of Orange G, Methylene blue and Rhodamine b textile dyes (0.05gm/ 100mL) in Nutrient agar broth by *Aspergillus flavus* was optimized. Among the four dyes tested the dye Methylene blue showed maximum degradation which compared to other Reactive dyes. The results indicated the bacteria showed maximum 90.6±0.52% decolorization of Methylene blue, 60±0.81% decolorization of Orange G and 55.5±0.61% decolorization of Rhodamine b after 5 days of incubation at 35°C in medium with high carbon and nitrogen sources. Our current study adds to attempts to comprehend the phenomenon of bacterial remediation of reactive dyes almost from laboratory circumstances to commercially application in field situations.

Keywords

Marine soil; isolation, *Aspergillus flavus*; textile dyes; biodegradation

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1. Introduction

In India, textile industries are the largest industries to provide employment for million people in the entire country. Worlds synthesis of textile yarns and fibres were contributed from India around 15%. In India national economy were decided by textile industries for 30% of the total exports and 14% of the total industrial production and the textile industries playing an important role in Indian economy (Castro et al. 2014). In dyestuff sector, the textile industry is interdependent. In textile industry nearly 75% of the dyestuff is produced and consumed by the textile industry (Kalyani et al. 2016). In textile industry the waste discharge was released and it contains toxic inorganic and certain organic compounds and these compounds are

carcinogenic and causes pollution to the environment. A few textile dyeing enterprises discharge chemicals tainted with dye into freshwater, which reduces light penetration and affects the photosynthetic rate of aquatic vegetation. The nutritional value of agricultural goods, as well as the health of animals and people, are negatively impacted by contaminated water that spreads pollutants to agricultural fields and causes skin rashes, blindness, chemosis, and dermatitis (Parmar et al. 2016 and Botsa et al. 2022).

Several wastewaters carrying various types of dyes are treated using a variety of phytoconstituents techniques, including filtration, co - precipitation, purification, chemical oxidation, aggregation, photodegradation, reduction, chemical flocculation, etc. These methods, however, are pricey, less effective, and produce a huge amount of sludge that is difficult to dispose of and, as a result, pollutes the soil. Microorganism-based biological methods are gaining popularity because they are inexpensive, produce less sludge, and are environmentally beneficial. Under specific environmental circumstances, microorganisms can totally mineralize and decolorize azo dyes, which are widely used in many industries. Moreover, certain commercial colours can be degraded by marine microbes. This research's objective is to identify microorganisms in coastal soil that can break down the dyes Methylene Blue, Orange G, and Rhodamine B.

2. Materials and Methods

Collection of sample

The soil sample was collected from Bay of Bengal, Vizag in sterile plastic containers with sterile spatula and transported to the laboratory as early as possible and stored at 4^{o}C before the experiment

Screening and isolation of fungi

The soil sample was diluted in sterile saline solution to a concentration of 1 g/9 ml. As part of a ten-fold serial dilution procedure, chloramphenicol was added to the media to prevent bacterial growth, and 0.1 mL of dilutions ranging from 10^{-1} to 10-^{9} were put onto PDA (Potato dextrose agar) plates. After inoculation, the plates were incubated at 30^{o}C for 72hr to detect fungi. After incubation the fungal colonies were observed. Morphologically different colonies were collected and again streaked on potato dextrose agar plates by using streak plate method, then plates were incubated at 30^{o}C for 72hr. The pure culture of fungi stored at 4^{o}C for further use.

Dye decolourization

The potato dextrose agar medium (50mL) were prepared separately containing the dyes (200 mg/l) and inoculated with 2% fungal culture. The conical flasks were incubated at 37ºC for one week. After incubation, the culture were centrifuged at 10000 rpm for 10 minutes and decolourization was assessed by spectrophotometer. The control flasks without dye were maintained as control. The percentage of dye decolorization was calculated as

$$\text{Decolorization (\%)} = \frac{(\text{Initial OD} - \text{Final OD}) \times 100}{\text{Initial OD}}$$

Optimization of dye decolorization

Decolorization of Orange G, Methylene blue and Rhodamine b textile dyes (0.05gm/ 100mL) in potato dextrose broth by *Aspergillus flavus* was optimized with respect to the effect of 1% nitrogen source (Beef extract, Yeast extract, Ammonium sulphate Sodium nitrate,), 1% carbon source (maltose, glucose, fructose, sucrose), pH (4.0, 7.0 and 9.0) and temperature (25, 35 and 45°C). One millilitre of fungal culture was used in each experiment, and PAD (Potato dextrose broth) without culture was used as the control. For 5 days, all of the flasks were incubated at 30°C with shaking.

3. Results and Discussion

Isolation of bacteria

PDA (Potato dextrose agar medium) was used for the isolation of fungi from marine soil sample During screening, four fungal isolates were selected based on their morphological characters. Based on the microscopic and morphological characters the selected fungi was named as *Aspergillus flavus.* Fig.1 showed the strain isolated from potato dextrose agar medium i.e. *Aspergillus flavus.*

Fig. 1: The isolated strain *Aspergillus flavus* on Potato dextrose agar medium.

Dye Decolorization of *Aspergillus flavus*

In potato dextrose broth *Aspergillus flavus* showed maximum decolorization. It showed 90.6±0.52% decolorization of Methylene blue, 60±0.81% decolorization of Orange G and 55.5±0.61% decolorization of Rhodamine B as shown in Fig.2. When compared to these three dyes Methylene blue has under gone maximum decolorization by *Aspergillus flavus* after 5 days of incubation (Figures 3 a-c). The main method of decolorization is dye adsorption on the surface of microorganisms (Knapp et al. 1995). It was also noted that *A. flavus* adsorbs dyes, and this was supported by the change in colour of the fungal mycelium in the colours that were observed. The ability of *F. solani* and *P. funigulosum* to decolorize Methylene blue (77%) and Crystal violet (60%) in solid media was demonstrated (Al-Jawhari et al. 2015). The efficiency of *A. flavus* in the degradation of Bromophenol blue (23%) and Congo red (33%) had already been investigated (Lokendra Singh et al. 2017 and Ved pal singh et al. 2022) which outfits that this fungus has the ability to be used as biodegrading agent. When compared to these findings *A. flavus* has more ability to degrade toxic dyes.

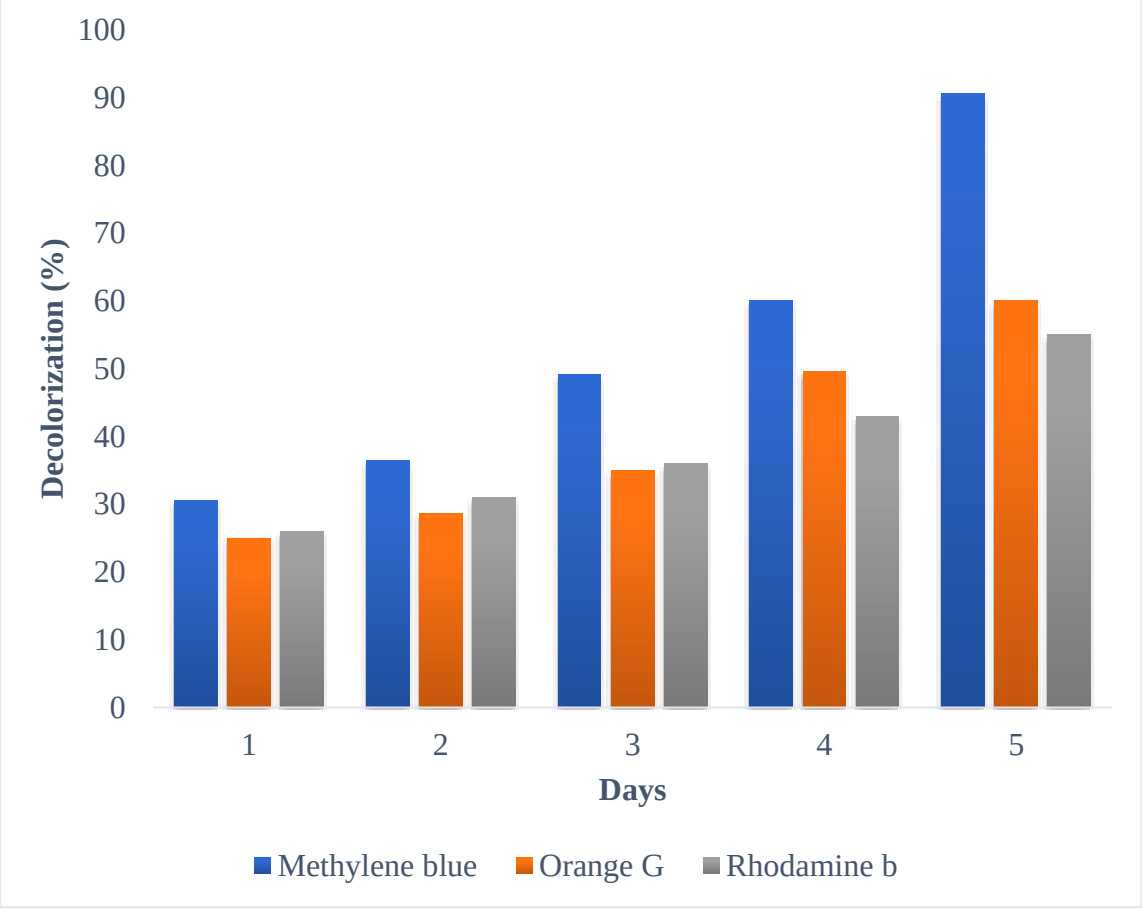

Fig. 2: Dye decolorization of Methylene blue, Orange G and Rhodamine b by *Aspergillus flavus*

Fig. 3: Dye decolorization by *Aspergillus flavus* over (a) Methylene blue (b) Orange G and (c) Rhodamine B

Optimization of conditions for Dye decolorization

For the optimization of decolorization of the Methylene blue, Orange G and Rhodamine b textile dyes, by *Aspergillus flavus*. All the experiments were conducted with different carbon and nitrogen sources, pH and temperature.

Effect of temperature

Experiments were conducted at a range of temperatures (25–45°C) while maintaining the same other parameters (pH 5.6, dye concentration of 0.05 g/100 mL). Maximum dye decolorization of Methylene blue (91±0.57%), Orange G (72±0.81) and Rhodamine b (690.57%) was observed at 35°C (Fig. 4). This finding was in consistent with previous studies reported by (Kannan et al. 2011), where the best decolorization of cationic dye was observed by *Aspergillus* sp. at temperature 35°C and 40°C with 94.25% and 83.65% decolorization respectively in 48 hr. The maximum dye decolorization of Congo red (86%) was reported at 31°C by *Aspergillus niger* and *Aspergillus flavus*, after the incubation period of five days under shaking conditions (Mohan et al. 2015).

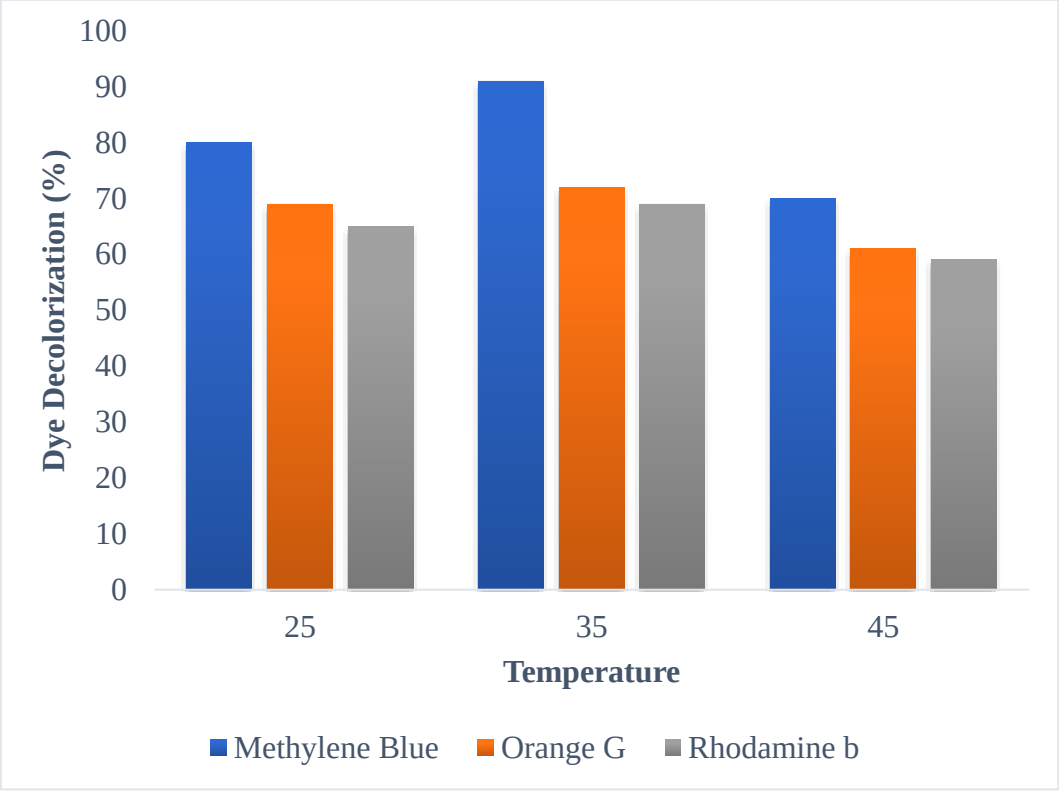

Fig. 4 Effect of temperature on % decolorization of dyes

Effect of pH

Effect of pH was investigated, keeping other parameters constant (temperature 35°C and dye conc. 0.05gm/100mL). Optimum decolorization of dye Methylene blue (92.1±0.17%)

was found at pH 7.0, with further decrease 85% at pH 9.0 respectively. Maximum dye decolorization of Orange G (81.83±0.76%) and Rhodamine b (79.1±0.76%) observed at pH 7.0 (Fig. 5) and the decolorization of dyes were decrease at pH 9.0 respectively. Praveen Sharma et al. 2010 reported that *B. subtilis* and *A. xyloxidans* are more efficient for maximum decolorization of Disperse Yellow 211 (80%) at pH 7.0. (Pratiksha Pradhan et al. 2012) reported maximum degradation (52.4, 59 and 50.7%) was observed for Congo red, Methyl orange and Malachite green, respectively at pH 7.0 by *Bacillus sp.* (Santhi et al. 2015) reported that *Pseudomonas aeruginosa* MTCC 424 was more efficient for maximum decolorization (76.8%) of Malachite green at pH 7.0.

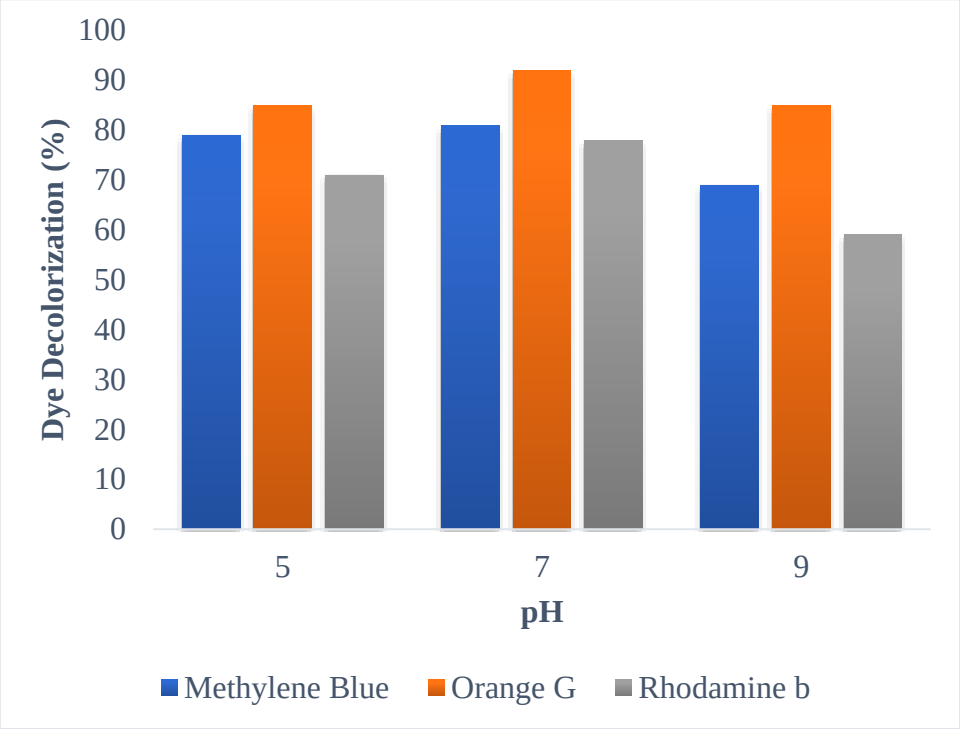

Fig. 5 Effect of pH on % decolorization of dyes

Effect of Carbon Source

The effects of different carbon sources (maltose, glucose, sucrose, and fructose) were investigated in trials under the same standard conditions (pH 7.0, dye concentration of 0.05 g/100 mL, and temperature of 35 °C). There was an increase in Methylene blue degradation up to 95.16±0.28% with glucose, Orange G dye degradation up to 89±0.47% with maltose and Rhodamine b dye degradation up to 62.16±0.28 with maltose (Fig. 6). When compared these two dyes Methylene blue has undergone maximum dye degradation up to 95.16±0.28. The present study revealed that addition of 1% glucose for Methylene blue and 1% of maltose for Orange G and Rhodamine b in the medium improved the colour removal (Jang et al. 2007) reported the 1% glucose increased the decolorization of Reactive dyes by *Citrobacter* sp.

Saraswati et al. 2009 had also reported the best growth and decolorization of Cibacron Black PSG (75%) by *B.cereus* was observed when lactose was used as carbon source. (Mohan et al. 2015) had also reported that growth and decolorization of congo red (93.21%) by *A. niger* and *A. flavus* in maltose as a carbon source.

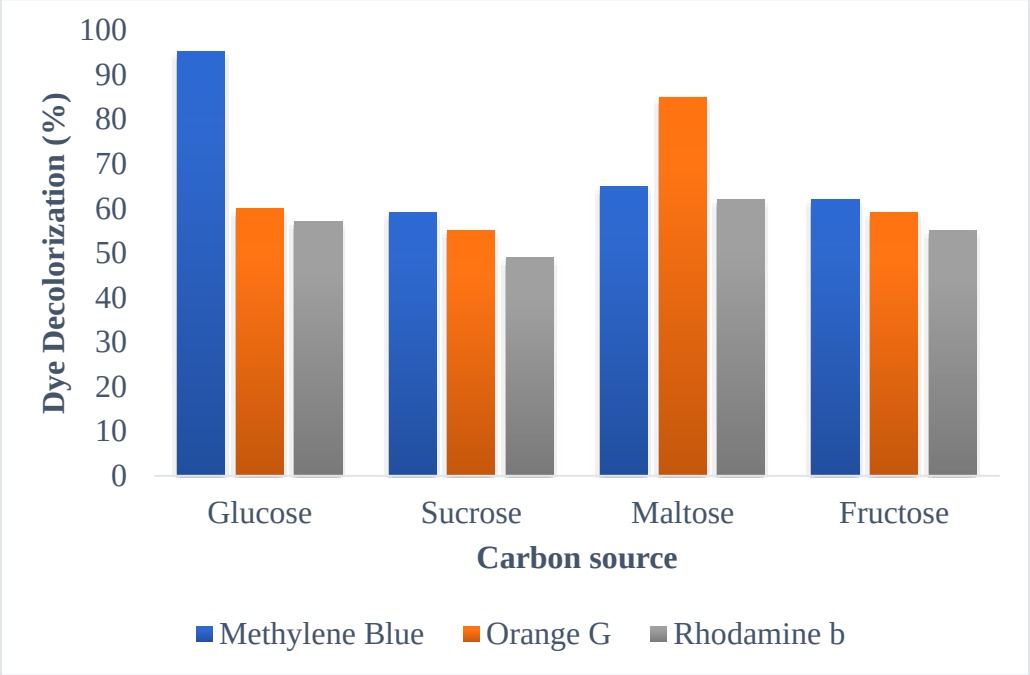

Fig. 6 Effect of carbon source on % decolorization of dyes

Effect of Nitrogen Source

Experiments were performed with different nitrogen sources (Yeast extract, Beef extract, Sodium nitrate, Ammonium sulphate) keeping other conditions constant (pH 7.0, dye conc. 0.05gm /100mL, Temp 35^{o}C). There was an increase in Methylene blue, Orange G and Rhodamine b dye degradation up to 97.1±0.17%, 90.83±0.76% and 87.1±0.17% (Fig.7) respectively after 5 days of incubation. Methylene blue, Orange G, Rhodamine b exhibited maximum degradation with 1% yeast extract. When yeast extract was employed as a nitrogen source, (Amith et al. 2012) noticed that *Aspergillu*s strain (MMF3) showed the highest percent decolorization, recording 86% of the Orange G dye. When yeast extract was employed as a nitrogen source, *P. chrysogenum* showed the highest percentage decolorization, recording 90% decolorization of the red 3BN dye, according to Praveen et al. 2012 ‘s observations. Moreover, *A. niger* and *A. flavus* were found to grow and decolorize Congo red (90%) when used in yeast extract as a nitrogen source, according to (Mohan et al. 2015).

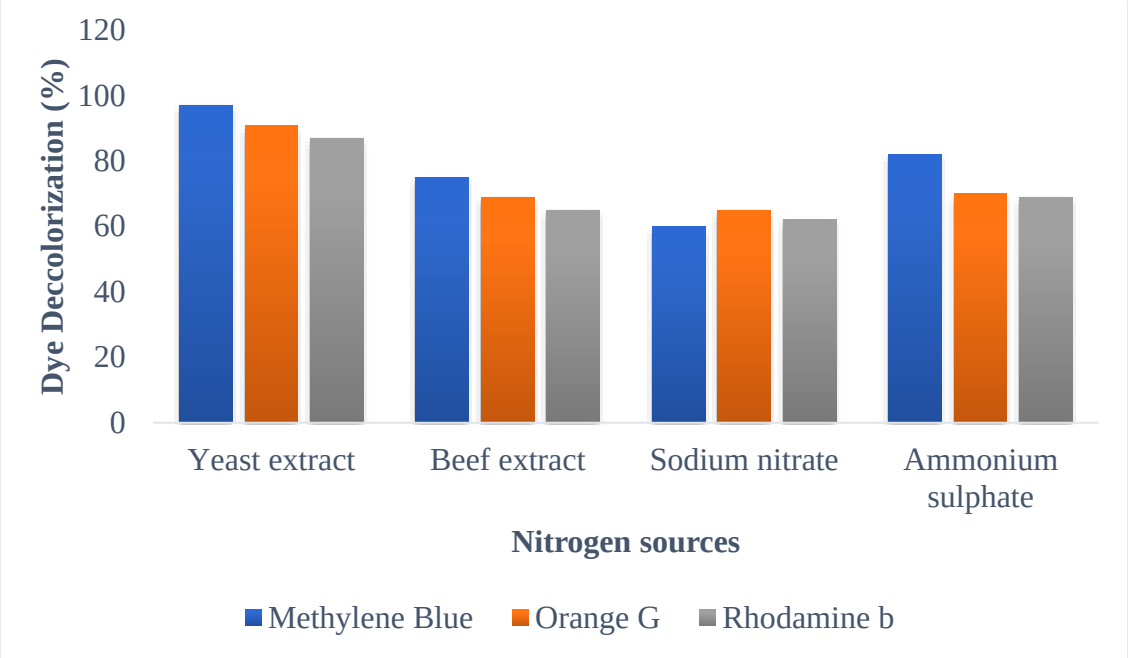

Fig. 7 Effect of nitrogen source on % decolorization of dyes

4. Conclusion

The original colour of many commercial products has been replaced with synthetic dyes as a result of increased industrialization. The textile, tanning, leather, printing, and many other sectors employ synthetic dyes. Several companies utilise synthetic colours extensively, which severely pollutes the water and is hazardous to microorganism existence. After physical and chemical treatment, companies discharge toxic effluent into the environment. Yet, a lot of industries either don't treat their effluent at all or only treat it partially physically and chemically. By introducing bacteria to cheaply breakdown the remaining colours in the effluent, these issues can be minimised. It is possible to use the potential of solitary dye-degrading bacteria in the bioremediation of such contaminants. The isolated Aspergillus flavus exhibited a great decolorization effectiveness (90.60.52%), according to our current investigation. This strain is suggested for use in textile dye decolorization. Methylene blue, one of the four dyes tested, degraded the fastest compared to the other Reactive dyes. The study makes a contribution to efforts to understand the phenomenon of bacterial remediation of reactive dyes almost from laboratory to commercially applicable in field situations.

References

Al-Jawhari I.F.H., 2015. Decolorization of methylene blue and crystal violet by some filamentous fungi. Int J Envirom Bioremd Biodeg., 3(2)., p.62-65.

Amit Kumar Singh., Ritu Singh., Akanksha Soam. and Sushil Kumar Shahi., 2012. Degradation of textile dye Orange 3R by *Aspergillus* strain (MMF3) and their culture optimization. Cur Dis Int J Cur Dis Inn., 1(1)., p.7-12.

Botsa S. M., Seetharam P., Raju, I. M., Suresh P., Satyanarayana G., Sambasivam S. and Tejeswararao D., 2022. Nanohybrid material of Co–TiO_2 and optical performance on methylene blue dye under visible light illumination. Hybrid Advances., 1., p.100001-100008.

Castro E., Avellaneda A. and Marco p.,2014. Combination of Advanced Oxidation Processes and Biological Treatment for the Removal of Benzidine-Derived Dyes. [J]. Environ Prog Sustain Energy., 33(3)., P. 873-85.

Jang MS., Jung BG., Sung NC. and Lee YC., 2007. Decolorization of textile plant effluent by *Citrobacter* sp. strain KCTC 18061P. J Gen Appl Microbiol., 53(6)., p339-343.

Kalyani P., Geetha S., Hemalatha V., Chandana Vineela K. and Hemalatha KPJ., 2016. Bioremediation of chromium by using *Aspergillus niger* (MTCC-961). World J Pharm Pharm Sci., 5(10)., p.881-885.

Knapp JS., Newby PS. and Recee LP., 1995. Decolourization of dyes by wood-rotting basidiomycete fungi. Enzyme Microb Technol., 17(7)., p.664-668.

Lokendra Singh. and Ved Pal Singh., 2017. Decolourization of Azo (Acid Red) and Anthraquinonic (Basic Blue) dyes by the fungus *Aspergillus flavus*. Int J Biol Eng Cli Sci., 3(1)., p.1-5.

Mohan SK., Arunkumar C., Perumal R., Venkatesaprabhu S. and Kavithanjali T., 2015. Optimization studies on the biodegradation of textile dye congo red using fungal strains. IJRASET., 3(1)., p.169-172.

Parmar P., Ojha VP. and Subramanian RB., 2016. Optimization of fusaric acid production by *Fusarium oxysporum* f. Sp. lycopersici using response surface methodology. Indian J Sci Technol., 3(4)., p. 411-416.

Pranali Jadhav. and Kishori Tarfe., 2019. Isolation of Methylene Blue dye degrading bacteria from effluent, screening and evaluation of their dye decolorization activity. International Journal of Scientific Development and Research (IJSDR)., 4(8)., p.224-230.

Pratiksha P., Harsh DK. and Gireesh Babu K., 2012. Degradation of Azo and Triphenylmethane dye by the bacteria isolated from local industrial waste. Int J Cur Res Rev., 4 (20)., p.39-49.

Praveen shrama., Singh L. and Dilbaghi N., 2010. Optimization of process variables for decolorization of disperse yellow 211 by *Bacillus subtilis* using Box-Behnken design. J Hazard Mater., 30(2-3)., p.1024-1029.

Santhi R. and Nalini sundhari MS., 2015. Optimization of malachite green decolourization by *Pseudomonas aeruginosa* MTCC 424. The Pharma Innovation Journal., 3(11)., p.11-15.

Saraswathi K. and Balakumar S., 2009. Biodecolourization of azodye (pigmented red 208) using Bacillus firmus and Bacillus laterosporus. J. Biosci. Technol., 1., p.1-7.

Shobina Kannan,, Jegathambal Palanichamy., Sugitha C., Mayilsami., 2011. Bioremediation of textile dyeing industry effluent from small scale industries using a microbial consortium of Bacillus sp., Escherichia coli, and *Aspergillus niger*. Journal of Applied Biology & Biotechnology., 10(2)., p.100-106.