Original Article

MOLECULAR CHARACTERIZATION OF DYE DEGRADING *Aspergillus flavus* STRAIN GKRS09 AND FT-IR ANALYSIS OF THE DEGRADED PRODUCTS

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Abstract

Azo dyes have aromatic rings, sulfonic groups, and azo bonds (-N=N-) making it one of the most often used dye classes in industry and a substantial class of environmental pollutants. The majority of textile dyes are flushed away through drainage systems. Due to their persistent nature and toxicity to both humans and animals, these dyes must be removed from the environment. The current work focuses on the biodegradation of such azo dye, Malachite Green (MG) and Congo Red (CR), by potent dye degrading fungal strain FSS4 isolated from textile water in Dravyavati river, Rajasthan, India. Potato dextrose broth with 100 mg/l MG and CR was used for dye degradation assay. FTIR (PerkinElmer Spectrum Two™ IR spectrometer) examined the degradation products from 4000–400 cm^{-1} wavenumber range. Molecular characterization identified this potent fungus as *Aspergillus flavus* strain GKRS09 (GenBank accession number; OK236565). The strain demonstrated remarkable decolorization and degradation properties after 07 days. FT-IR study showed that *A. flavus* strain GKRS09 decomposed Malachite Green and Congo Red dyes into non-toxic products. The isolate GKRS09 can treat azo dye-containing industrial effluent economically and sustainably.

Keywords: Textile dye, Environment, Health, Degradation, Fungi, Water

1. Introduction

Water pollution is a prominent problem among the numerous forms of environmental contamination. The color of the water is one of the most visible signs of pollution. Clothing dyes are emitted into industrial effluents in the amount of 280,000 tons per year (Sheam et al. 2021). Synthetic dyes are xenobiotics that exhibit recalcitrance (Sosa-Martinez et al. 2020). On a commercial scale, over bout 100,000 different varieties of synthetic pigments and dyes are used. Azo dyes have one, two, or even three azo bonds (R1–N=N–R2) in their structures. These dyes make up sixty to seventy percent of all synthetic dyes that are manufactured (Almeida and Corso 2019). Congo red has two azo linkages (-N=N-) chromophores and resistant to biodegradation (Asses et al. 2018). Textile industry discharge of leftover colors (azo dye), dispersing agents, salts, and heavy metals which may limit aquatic photosynthesis, decrease dissolved oxygen, and harm people and other species (Saroj et al. 2015).

Physicochemical treatments for textile wastewater are costly, energy-consuming, create dangerous byproducts, and are less successful with a broad variety of dyes (Wesenberg et al. 2003; Oliveira et al. 2020). Recently,

biological methods of dye decolorization have gained popularity since they are inexpensive and may be used in a broad variety of dyes (Dexilin et al. 2021). Fungal systems (mycodecolorization) are best for treating textile dye effluents (Chang et al. 2001; Balaji et al. 2012). Fungal isolates biosorb and biodegrade toxic dyes. Several fungi, such as *Aspergillus flavus* (Esmaeili and Kalantari 2012), *Aspergillus terreus* (Singh and Dwivedi 2020), and *Aspergillus niger* (Asses et al. 2018) have been reported as potential biodegrades of pollutant compounds.

The present work details the isolation and screening of fungal strains from dye-contaminated soil and water, as well as the, evaluated the dye degradation capacity of the fungal strain. The degradation of the dyes was further investigated using Fourier transform infrared spectroscopy. As a result of the findings, it can be inferred that *Aspergillus flavus* strain GKRS09 may be utilized to decolorize textile dye effluents in an environmentally benign and economically viable manner.

2. Materials and methods

Chemicals and Dye Stuff

Malachite Green (CAS No.: 569-64-2) (Figure 1) and Congo Red (CAS No.: 573-58-0) (Figure 2) were purchased from Dye industry, Jaipur, Rajasthan, India. All chemicals were analytical grade and bought from HiMedia (India), Thermo Scientific (Hampshire, UK), and Sigma (St. Louis, MO, United States).

Figure 1: Malachite Green's Chemical Structure

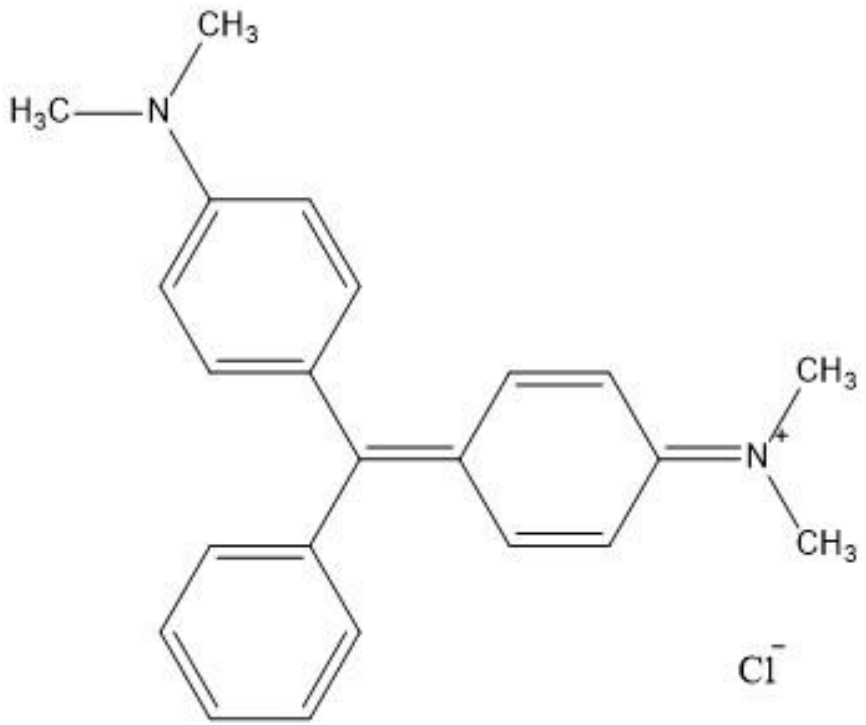
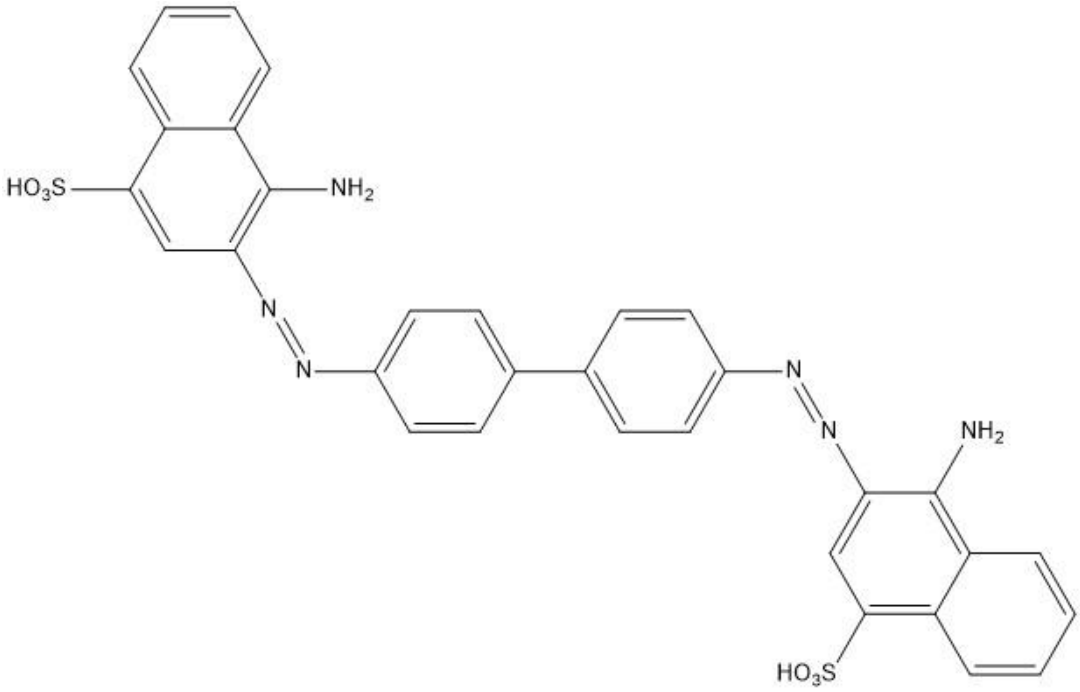


Figure 2: Congo Red's Chemical Structure



Collection of Soil and Wastewater from the Dravyavati river

The samples were collected from the Dravyavati river at Sanganer industrial area located in Jaipur, Rajasthan, India for isolation of fungal strains (Photograph 1). Samples were put in sterile glass tubes with screw-on caps and kept at 4°C. They were tested within 24 hours of being taken.

Photograph 1: Collection of soil and water samples from textile sludge

Isolation of Dye Degrading Fungi

The 200 mg L-1 broth MSM enriched with glucose (0.2 % w/v) was used (Khalid et al. 2008) and spiked with malachite green and congo red dye (100 mg L^{-1}). 10 mL wastewater and 1 g soil inoculated into mixture. The flasks were incubated at 30°C for 7 days on an orbital shaker at 120-rpm. After 3 and 7 days, cell suspensions from each flask were plated onto Potato Dextrose Agar (PDA) medium and incubated at 28°C for 7 days. The pure culture of fungal strain was maintained for the dye degradation assay. The strain was also stored at the Biotechnology laboratory of JECRC University.

Screening of Fungi for Dye Degradation

A disc of fungal mycelium 6 mm in diameter was put in the center of Petri plates with PDA media enriched with Malachite green and Congo red dye at a concentration of (0.01 %). The plates were incubated at 28°C for 7 days. Triplicated experiments were performed. The colony diameter and decolorization zone were measured (Machado et al. 2005).

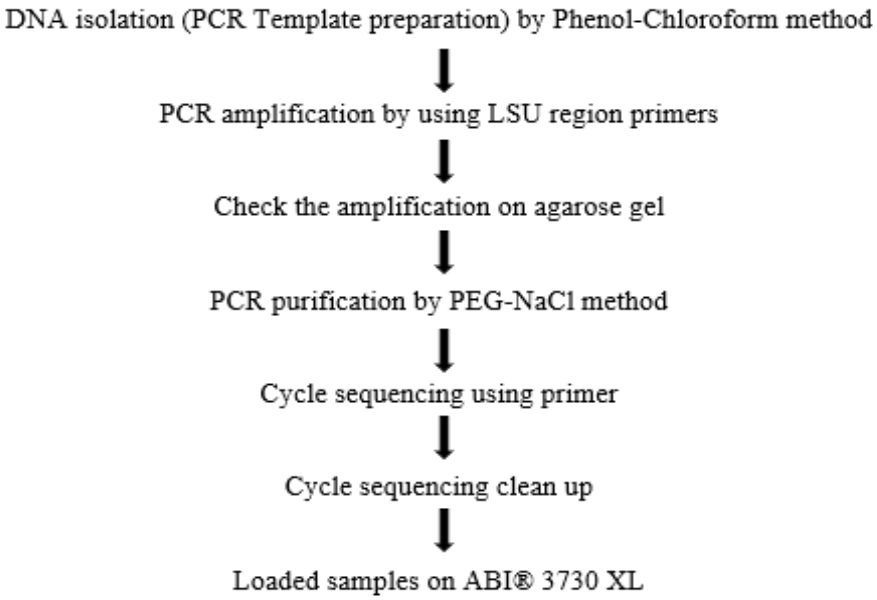
Preliminary Identification of the Isolated Fungal Strain

The Lactophenol Cotton Blue (LPCB) wet mount method was used to identify fungi. The fungi were observed through a microscope on both low and high magnification (Olympus Light Microscope). Laboratory Methods in Basic Mycology (Forbes et al., 2000) and Pictorial Atlas of Soil and Seed Fungi (Watanabe 1937) were used for identification.

Molecular Characterization of Dye Degrading Fungi

The NCMR National Centre for Microbial Resource (NCMR) sequencing facility in Pune identified the fungal isolate. The facility extracted genomic DNA using the conventional phenol/chloroform extraction procedure (Sambrook et al. 1989) and PCR amplified the LSU regions using universal primers LROR [5'-ACCCGCTGAACTTAAGC -3'] and LR5 [5'- TCCTGAGGGAAACTTCG -3']. The amplified PCR product sequenced on an ABI® 3730XL automated DNA sequencer (Applied Biosystems, Inc., Foster City, CA) (Boratyn et al. 2013).

General sequencing protocol



NCBI Gene Bank Deposition

Aspergillus flavus GKRS09's nucleotide sequence was deposited in the NCBI gene bank under accession number OK236565.

Evolutionary relationships of taxa

Neighbor-Joining inferred the evolutionary history (Saitou and Nei 1987). The Maximum Composite Likelihood technique (Tamura et al. 2004) calculated evolutionary distances in base substitutions per site. MEGA11 used to construct phylogenetic tree (Tamura et al. 2021).

Biodegradation Studies

A dye degradation experiment was done in potato dextrose broth supplemented with 100 mg/l Malachite green and Congo Red inoculated with *Aspergillus flavus* biomass (8mm disc) incubated at 30°C for 7 days on an orbital shaker at 120 rpm. Control flasks were unsupplemented.

Analytical procedure (UV-Visible and FTIR analyses)

The absorbance at 497 nm on a UV/VIS spectrophotometer was used to determine the extent of colour reduction. The absorbance differences in the supernatants were measured after centrifuging the cultures at 5000 rpm for 15 minutes. The proportion of decolorization achieved from the biodegradation experiments was calculated using the following formula (Almeida and Corso, 2014):

$$\boldsymbol{Decolorization}\ (\%) = \frac{(A0-A1)}{A0}\ X\ 100 \text{.....(i)}$$

A0 represents the dye absorbance before decolorization, whereas A1 represents the dye absorbance after decolorization.

The functional group characterization of the dyes before and after decolorization was studied. The degraded products were analyzed over the wavenumber range of 4000–400 cm^{-1} by FTIR (PerkinElmer's Spectrum Two™ IR spectrometer) at Central Instrumentation Facility, Lovely Professional University, Punjab, India.

3. Results and discussion

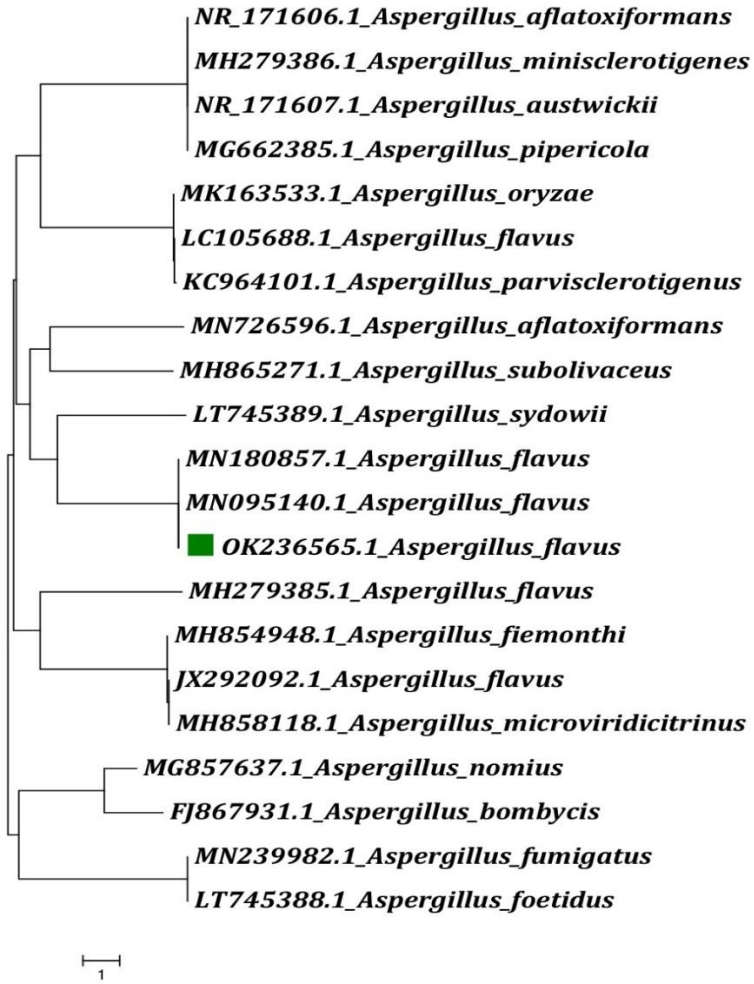
Aspergillus flavus was isolated from textile effluent. The isolated fungal species were preliminary identified at the Department of Biotechnology at JECRC University, INDIA (Photograph 2). It was also identified using 18s rRNA sequencing.

Photograph 2: Microscopic characteristics of *A. flavus* GKRS09 under 40× magnification

The isolated fungus was tested for the decolorization activity of Congo red and Malachite green dye. Single microbial strains may decolorize dyes, although the metabolites produced are often more difficult to biodegrade than the dye itself. Due to the varied composition of textile wastewater, numerous research groups have sought to create more effective microbial methods.

The isolated fungi showed a 16 ± 0.2 mm zone of clearance diameter on the malachite green enriched PDA plate and 14 ± 0.2 mm on the congo red enriched plate. After preliminary screening of dye colorization, the potent fungi were identified under a light microscope and confirmed as *Aspergillus*. For species-level identification, the fungus was molecularly characterized. The 18S rRNA sequence data of *Aspergillus flavus* deposited to GenBank was assigned accession numbers OK236565. Phylogenetic analysis showed the organisms' genera (Figure 3).

Figure 3: Phylogenetic tree of *Aspergillus flavus* strain GKRS09 based on 18S rDNA analysis

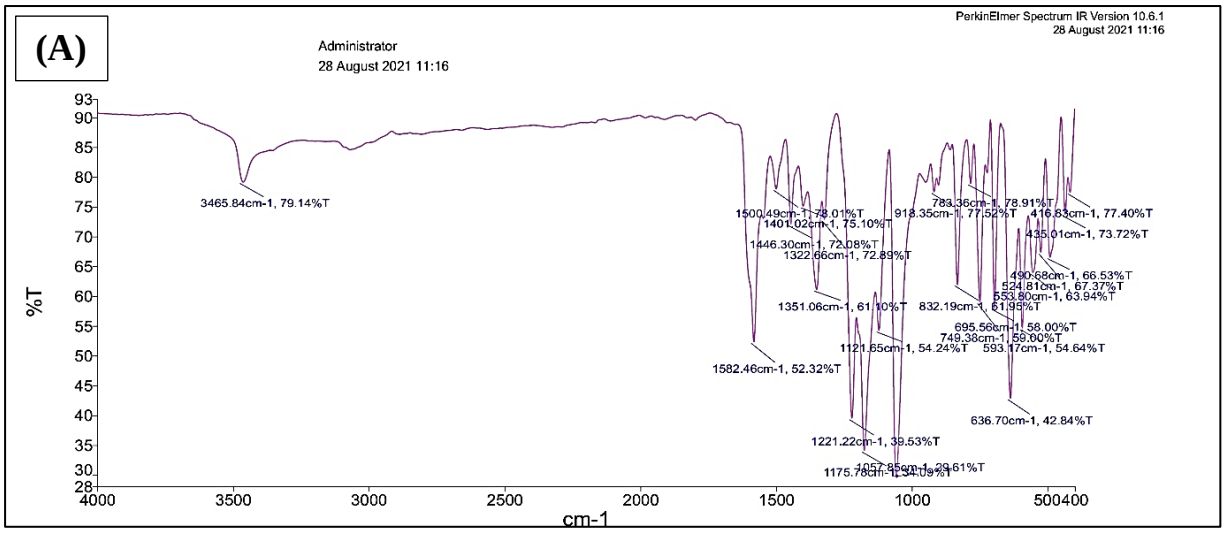


Azo dyes are ubiquitous environmental pollutant that resists biodegradation and has adverse biological consequences. Thus, novel microbiological agents and ecologically friendly, cost-effective textile effluent treatment techniques are essential. This work investigates the bioremediation of synthetic azo dyes by *Aspergillus flavus* isolated from textile dye-contaminated soil. Congo Red (CR) and Malachite Green (MG) color removal was confirmed by UV-visible analysis. Decolorization of dyes in the medium by *Aspergillus flavus* GKRS09 is usually done by adsorption of dyes on fungal mycelia or biodegradation by enzymes. After 7 days of incubation at 30°C and 120 rpm batch-culture conditions, 100 mg/L dye concentration in potato dextrose broth decolorized CR 89.28% and MG 93.44%. Sheam et al. 2021 reported that *Aspergillus salinarus* stains can destroy 97.41% of Reactive Red HE7B dye in potato dextrose broth with 50 mg/l RR dye. Additionally, this dye-degrading fungus stain was detected using ITS sequencing.

Balaji et al. 2012 studied the decolorization capabilities of *Aspergillus niger* against the azo dye (Red HE7B) on potato dextrose agar medium under static in-vitro conditions and found the highest percentage of degradation (94%) after 5 days of incubation. Singh and Dwivedi, 2021 identified Congo red (CR) dye decolorizers Aspergillus terreus GS28 and *Aspergillus flavus* CR500 from industrial waste sludge. *A. terreus* decolorizes CR (95.0%) better than *A. flavus* (92.96%) after 120 h under optimum circumstances. Filamentous fungus decolorize well at pH 6–7 (Subramanian et al. 2014). *Aspergillus flavus* is found in synthetic dye-contaminated areas and degrades dyes, according to prior investigations (Lalitha et al. 2011).

The FTIR spectrum comparison between the control dye sample and the samples extracted following decolorization by Aspergillus flavus strain GKRS09 confirmed Congo Red (CR) and Malachite Green degradation (MG). FTIR study showed spectra peak decrease (Figures 4b and 5b). These peaks moved between the spectrums of control and treatment dyes. Shifting these peaks showed dye degradation (Figures. 4a, b, and 5a, b). Previous studies found similar peak shifts in dyes (Koyani et al. 2013).

Figure 4: FT-IR spectra of (a) control Congo Red and (b) its degradation metabolites.



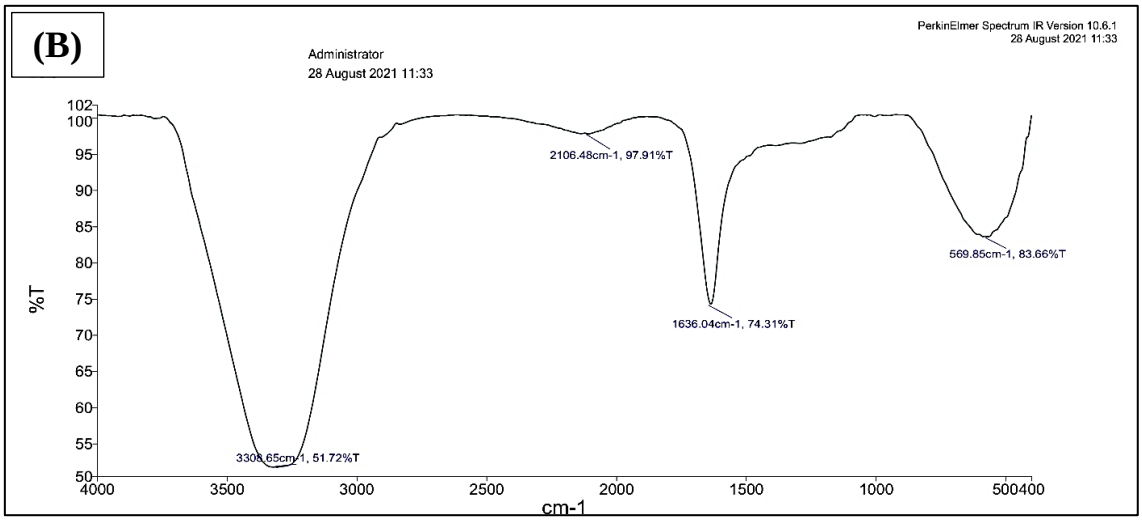
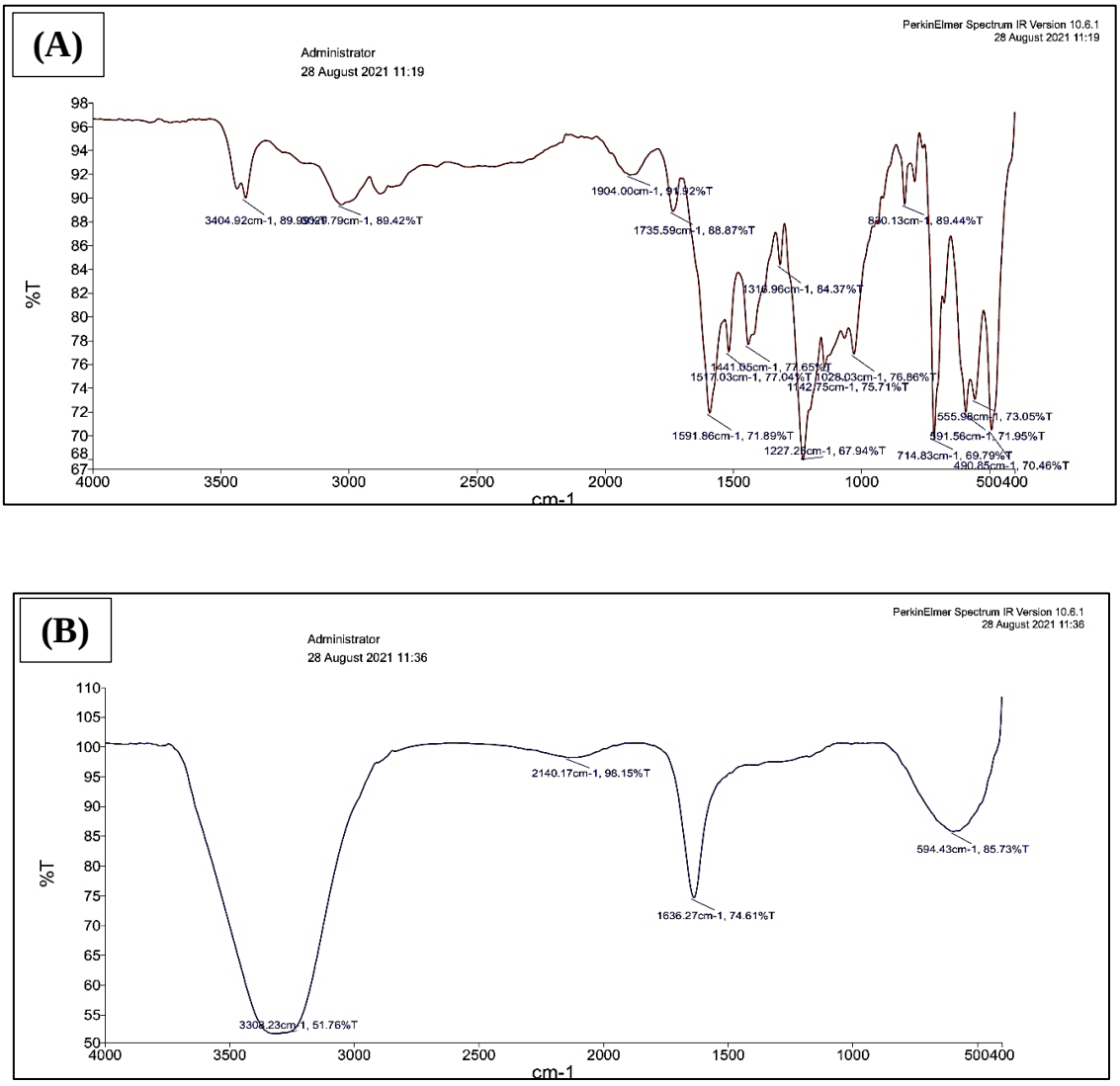


Figure 5: FT-IR spectra of (a) control Malachite Green and (b) its degradation metabolites.



4. Conclusion

A fungal strain, *Aspergillus flavus* strain GKRS09, was shown to be capable of decomposing malachite green and Congo Red as a single source of carbon with minimum nutritional needs when grown in a static environment. Because of its capacity to decolorize malachite green and congo red across a broad range of pH, temperature, salt, and starting dye concentrations, this strain has the potential to be used in a variety of commercial applications. The findings of the FT-IR analysis revealed that the *A. flavus* strain GKRS09 destroyed the aromatic character of the dyes malachite green and congo red. *A. flavus* strain GKRS09 is a very promising bacterium that has the potential to be employed in the treatment of textile industry effluents containing different malachite green and congo red dyes.

Conflict of interest

The authors state that no conflicts of interest exist.

Acknowledgments

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References

1. Almeida, E. J. R. and Corso, C. R. 2014. Comparative study of toxicity of azo dye Procion Red MX-5B following biosorption and biodegradation treatments with the fungi *Aspergillus niger* and *Aspergillus terreus*. Chemosphere., 112 : 317–322.

2. Almeida, E. J. R. and Corso, C. R. 2019. Decolorization and removal of toxicity of textile azo dyes using fungal biomass pelletized. Int. J. Environ. Sci. Tech., 16(3) : 1319–1328.

3. Asses, N. Ayed, L. Hkiri, N. and Hamdi, M. 2018. Congo Red Decolorization and Detoxification by *Aspergillus niger* : Removal Mechanisms and Dye Degradation Pathway. Biomed Res. Int., 2018 : 1–9.

4. Balaji, V. Vinayagamoorthi, D. Palanisamy, A. and Anbalagan, S. 2012. Degradation of Reactive Red HE7B and Yellow FN2R dyes by fungal isolates. J. Acad. Indus. Res., 1(3) : 132-136.

5. Boratyn, G. M. Camacho, C. Cooper, P. S. Coulouris, G. Fong, A. Ma, N. Madden, T. L. Matten, W. T. McGinnis, S. D. Merezhuk, Y. Raytselis, Y. Sayers, E. W. Tao, T. Ye, J. and Zaretskaya, I. 2013. BLAST: a more efficient report with usability improvements. Nucleic Acids Res., 41(W1) : W29–W33.

6. Chang, J. S. Chou, C. and Chen, S. Y. 2001. Decolorization of azo dyes with immobilized *Pseudomonas luteola*. Process Biochem., 36(8–9) : 757–763.

7. Dexilin, M. Gowri Manogari, B. and Kaliannan, T. 2021. Optimization of Culture Conditions for Enhanced Decolorization of Azo Dyes by *Aspergillus flavus* Isolated from Dye Contaminated Soil. In: Marimuthu, P.D. Sundaram, R. Jeyaseelan, A. Kaliannan, T. (eds.) Bioremediation and Green Technologies. Environmental Science and Engineering. Springer, Cham.

8. Esmaeili, A. and Kalantari, M. 2012. Bioremoval of an azo textile dye, Reactive Red 198, by *Aspergillus flavus*. World J. Microbiol. Biotechnol., 28(3) : 1125–1131.

9. Forbes, B. A. Sahm, D. F. and Weissfeld, A. S. 2002. Laboratory methods in basic mycology. Bailey & Scott's Diagnostic Microbiology, 11th edn. Mosby: Inc., pp.711-797.

10. Khalid, A. Arshad, M. and Crowley, D. E. 2008. Accelerated decolorization of structurally different azo dyes by newly isolated bacterial strains. Appl. Microbiol. Biotechnol., 78(2) : 361–369.

11. Koyani, R. D. Sanghvi, G. V. Sharma, R. K. and Rajput, K. S. 2013. Contribution of lignin degrading enzymes in decolourisation and degradation of reactive textile dyes. Int. Biodeterior. Biodegr., 77 : 1–9.

12. Lalitha, P. Reddy, N. N. R. and Arunalakshmi, K. 2011. Decolorization of Synthetic Dyes by *Aspergillus flavus*. Bioremediat. J., 15(2) **:** 121–132.

13. Machado, K. M. G. Matheus, D. R. Monteiro, R. T. R. and Bononi, V. L. R. 2005. Biodegradation of pentachorophenol by tropical basidiomycetes in soils contaminated with industrial residues. World J. Microbiol. Biotechnol., 21(3) : 297–301.

14. Oliveira, J. M. S. de Lima e Silva, M. R. Issa, C. G. Corbi, J. J. Damianovic, M. H. R. Z. and Foresti E. 2020. Intermittent aeration strategy for azo dye biodegradation: A suitable alternative to conventional biological treatments?. J. Hazard. Mater., 385 : 121558.

15. Saitou, N., and Nei, M. 1987. The neighbor-joining method: a new method for reconstructing phylogenetic trees. Mol. Biol. Evol., 4(4) : 406–425.

16. Sambrook, J. Fritsch, E. F. and Maniatis, T. 1989. Molecular Cloning: A Laboratory Manual, Second Edition. Cold Spring Harbor Laboratory Press.

17. Saroj, S. Dubey, S. Agarwal, P. Prasad, R. and Singh, R. P. (2015). Evaluation of the efficacy of a fungal consortium for degradation of azo dyes and simulated textile dye effluents. Sustain. Water Resour. Manag., 1(3) : 233–243.

18. Sheam, M. M. Biswas, S. K. Ahmed, K. R. Syed, S. Bin, Hossain M. S. Khan, M. S. A. Hasan, M. R. Zohra, F. T. and Rahman, M. M. 2021. Mycoremediation of reactive red HE7B dye by *Aspergillus salinarus* isolated from textile effluents. Current Research in Microbial Sciences, 2 : 100056.

19. Singh, G. and Dwivedi, S. K. 2021. Biosorptive and Biodegradative Mechanistic Approach for the Decolorization of Congo Red Dye by *Aspergillus* Species. Bull. Environ. Contam. Toxicol., 108 : 457–467.

20. Singh, G. and Dwivedi, S. K. 2020. Decolorization and degradation of Direct Blue-1 (Azo dye) by newly isolated fungus *Aspergillus terreus* GS28, from sludge of carpet industry. Environ. Technol. Innov., 18 : 100751.

21. Sosa-Martínez, J. D. Balagurusamy, N. Montañez, J. Peralta, R. A. Moreira, R. de F. P. M. Bracht, A. Peralta, R. M. and Morales-Oyervides, L. 2020. Synthetic dyes biodegradation by fungal ligninolytic enzymes: Process optimization, metabolites evaluation and toxicity assessment. J. Hazard. Mater., 400 : 123254.

22. Subramanian, J. Ramesh, T. and Kalaiselvam, M. 2014. Degradation Of Triphenylmethane Dye: Malachite Green By *Aspergillus flavus*. World J. Pharm. Pharm. Sci., 3(8) : 44-50.

23. Tamura, K. Nei, M. and Kumar, S. 2004. Prospects for inferring very large phylogenies by using the neighbor-joining method. Proc. Natl. Acad. Sci. U.S.A., 101(30) : 11030–11035.

24. Tamura, K. Stecher, G. and Kumar, S. 2021. MEGA11: Molecular Evolutionary Genetics Analysis Version 11. Mol. Biol. Evol., 38(7) : 3022–3027.

25. Watanabe, T. 1937. Pictorial atlas of soil and seed fungi: morphologies of cultured fungi and key to species. 2nd edn. Boca Raton, Florida: CRC Press LLC.

26. Wesenberg, D. 2003. White-rot fungi and their enzymes for the treatment of industrial dye effluents. Biotechnol. Adv., 22(1–2) : 161–187.