

Evaluation of Azo Dye Degradation Potential of *Aspergillus niger* and *Penicillium simplicissimum*

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ABSTRACT

This study examines the potential of fungal strains for degrading azo dyes, providing an eco-friendly and efficient alternative to traditional methods. For the dye decolorization assay, two fungal strains were used to evaluate their degradation capability on Congo Red, yielding decolorization rates of 90% by *Aspergillus niger* and 91% by *Penicillium simplicissimum*, respectively, over 60 days. According to the decolorized dye's Gas Chromatography-Mass Spectrometry (GC-MS) examination, several dye-degraded products, including octadecanoic acid, benzoic acid, 3,4,5-trihydroxy, 9H-fluoren-9-one, and many others, were generated by the fungal strains. The azo dye-degradative compounds have potential applications as they possess antioxidant and antimicrobial properties. These fungal strains have promising potential to degrade toxic azo dyes into less hazardous compounds, as evidenced by dye-degraded products, and can be used to clean the environment from such toxic compounds/dyes.

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Authors' Contribution

DQ and MBKF performed experiments, analyzed the data and wrote the manuscript. AR helped in research design and in manuscript editing. All authors approved the final manuscript.

Key words

Azo dye, Fungal strains, GC-MS analysis, Wastewater, Bioremediation

INTRODUCTION

A number of environmental issues that negatively affect the soil, water, and biotic elements of the biosphere have been brought on by the persistent efforts to develop various industries, including the chemical, pharmaceutical, agricultural, and textile sectors (Dutta *et al.*, 2024). Azo dyes, the vibrant hues that are widely used in the textile, paper, and food industries, pose significant environmental and health risks due to their toxic and recalcitrant nature. Many contaminants persist in the environment and cause air, water and soil pollution and they are physical contaminants (plastic, heavy metals), chemical contaminants (dioxins, azo dyes, PAHs) or biological contaminants (bacteria, viruses etc.) (Ali *et al.*, 2023) and there are many methods to remove contaminants such as UV treatment, Filtration, Chemical precipitation and Distillation etc.

Dyes are widely used in many industries, and they

are classified as water-soluble dyes (Acidic, Basic, Direct, and Reactive dyes) and water-insoluble dyes (Disperse, Vat, and Sulfur dyes). These kinds are resistant and they impose negative impacts on human health as well as on plant growth, leading to body impairments and growth-related disorders.

Azo dyes have become essential in today's world because they have entered every aspect of life, as natural dyes are expensive and tiring to apply. They are a commercially important family of azo compounds, i.e., compounds containing the C-N=N-C linkage. So, the release of azo dyes into the environment has become a significant concern due to their toxic and carcinogenic properties and is responsible for ADHD in kids, inefficient immune response generation in humans, type 1 hypersensitivity reactions among individuals, etc.

For the azo dyes decolorization, several methods are employed, including physical methods (adsorption, coagulation, flocculation), which use physical forces to break down dye molecules, chemical methods (ozonation and advanced oxidation processes), and biological methods using microorganisms to degrade complex organic compounds into simpler compounds. Biological agents like fungi and bacteria are the most reliable way to break down dyes. Because living things like these either adsorb or absorb colors into their cells, they lack any toxic substances (Ilyas and Rehman, 2013). In the current

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investigation, two fungal strains i.e., *Aspergillus niger* and *Penicillium simplicissimum* are used to evaluate their potential to degrade azo dye.

MATERIALS AND METHODS

Fungus strains and culturing media

Two fungus strains i.e., *Penicillium simplicissimum* and *Aspergillus niger* were obtained from Pakistan's first Fungal Culture Bank (FCBP), University of the Punjab.

A natural potato dextrose agar was made for the cultivation of strains. A kilogram of peeled potatoes was boiled in 1000 mL of tap water; distilled water was added as needed. After that, 20g of agar was added and then autoclaved. After that, 10mL of glucose solution was added. After mixing thoroughly, 2 mL of the chloramphenicol stock (9 mL of 70% ethanol and 0.25g of chloramphenicol powder were combined, and the volume was raised to 10mL using autoclaved distilled water) was added. Mueller-Hinton broth was prepared by dissolving 2 g beef extract, 17.5 g tryptone, and 1.5 g starch in one liter of distilled water for further growth of fungal strains. The media were subject to incubation for three days (*A. niger*) and seven days (*P. simplicissimum*) at 30 ± 2 °C.

Fungal decolorization of dyes

After preparing and autoclaving the Mueller-Hinton medium (200 mL), it was then inoculated with both strains of the fungi, namely *Penicillium simplicissimum* and *Aspergillus niger*, and incubated until the flasks were fully cultivated. After incubation, 0.01% of Congo Red dye was added to both the flasks. After nearly two months, the preparations were removed and centrifuged for five minutes at 14500 rpm. At 550 nm, the optical densities of the test and control samples for Congo Red were measured.

The formula was used to determine the percentage of dye decolorization (Vithalani and Bhatt, 2023).

$$\text{Decolorization percentage (\%)} = \frac{\text{The difference between the initial and final absorbance}}{\text{Initial absorbance}} \times 100$$

Wastewater degradation by fungi

Textile wastewater was collected and inoculated with the *A. niger* strain. This preparation was kept for one month, taken out, centrifuged at 14500 rpm, and then the optical density of control and test samples was measured at 550nm, and their percentage decolorization was calculated.

GC-MS analysis

The flasks having decolorized dye and red wastewater decolorized by the fungal strains were taken. The fungal mat was removed, and 1 mL from each preparation was taken into an Eppendorf tube separately. These were

centrifuged at 14500 rpm for 5 min and finally underwent. For the examination of compounds produced by fungi, a Gas Chromatograph Mass Spectrometer from Agilent USA was used in conjunction with gas chromatography and mass spectrometry. With the test volume of 1 μ L, the inlet temperature was set at 280°C. Helium, with dimensions of DB 5MS 30m, 0.25mm, or 0.25 μ m, was included in the column and flowed at a rate of 1 mL/min. After being kept at 50°C for 1 min, the oven's temperature was progressively raised by 15°C each min or 320°C for 5 min. It ran for 24 min in total. The procedure lasted for roughly 4.8 min, with the transfer line temperature maintained at 280°C and the MS mode set to Scan (35–500).

RESULTS

Fungal dye decolorization

When the media containing the dye and fungi were examined after incubation, it was evident that the fungus strains enzymatic activity had caused a color shift from dark to light hues. The treated samples optical density values were lower than the control. *A. niger* degraded Congo Red by 90% within 60 days, whereas *P. simplicissimum* showed degradation upto 91% within 60 days of incubation (Fig. 1).



Fig. 1. Color change/degradation of azo dye in the presence of *A. niger* (left) and *P. simplicissimum* (right) as compared to the control.

GC-MS analysis of degradation product of Congo Red

Several compounds were produced by the action of *A. niger* when it degraded Congo Red, including Tetradecane, Eicosane, Nonadecane, 3-Methyl-2,3,6,7,8,8a-hexahydropyrrolo[1,2-a]pyrazine-1,4 dione, Octadecanoic acid, Benzoic acid, 3,4,5-trihydroxy, 9H-fluoren-9-one, 2,7-diamino, Ferrocene, (carboxyethynyl)-, trans-2,3,5-Trimethoxy-.beta.-methyl-.beta.-nitrostyrene, 5-Bromo 1-methylindole-2-carboxylic acid, and 1-benzylindole, etc. (Fig. 2A).

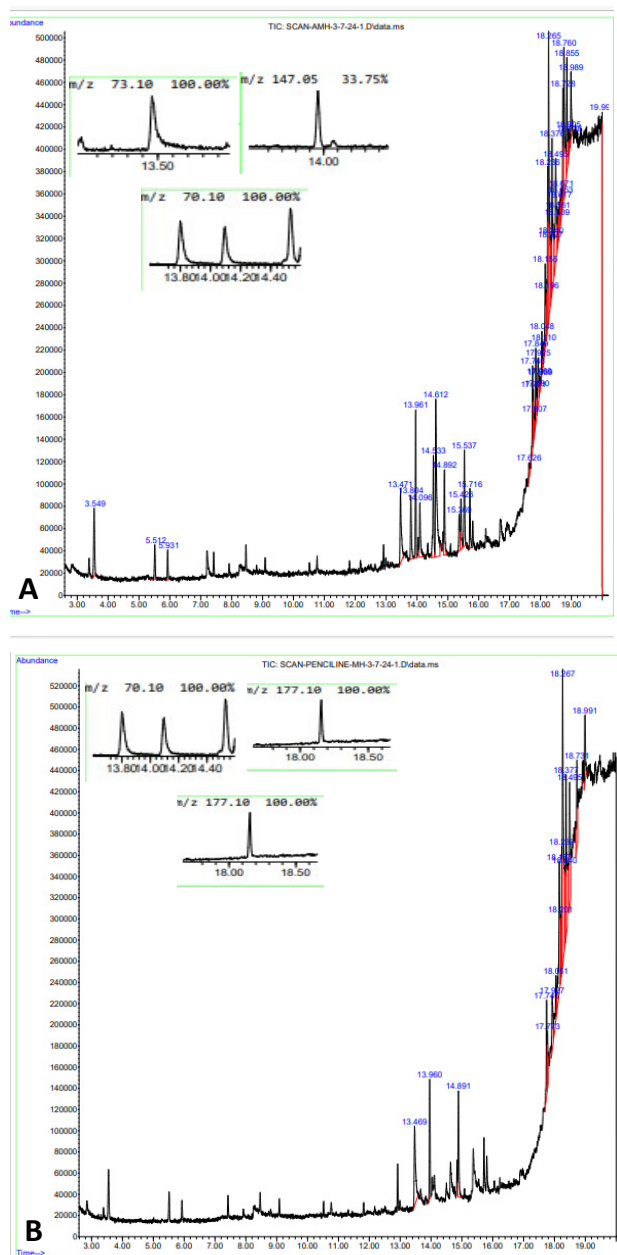


Fig. 2. Compounds produced by the action of (a) *A. niger* and (b) *P. simplicissimum* on Congo Red-containing medium.

The compounds produced by *P. simplicissimum* by its action on Congo Red included n Hexadecanoic acid, Benzene, 1-methoxy-4 (2-phenylethenyl)-, 1H-Pyrrolo[2,3-b]pyridine, 3-amino-2-(4-pyridyl)-, 9H-fluoren-9-one, 2,7-diamino,(2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, trans, 5-Phenylvaleric acid, 2-methyloct-5-yn-4-yl ester, Pimelic acid, di(2

chlorophenyl) ester, etc (Fig. 2B). A summary of all the compounds produced by both strains after comparing with control is given in Table I.

Wastewater degradation

Post-incubation, wastewater containing *A. niger* strain was observed, and there was a noticeable shift in hue from dark red to bright (Fig. 3). Fungal mat was removed; the sample was centrifuged at 14500 rpm and then the optical density of the control and test sample was taken and there was decrease in the absorbance value of the test sample. The percentage of decolorization was found to be 70%.



Fig. 3. Flask 1, containing only wastewater, acts as a control, whereas flask 2 contains decolorized wastewater treated with the fungal strain.

GC-MS analysis of degradation products of Congo Red by *A. niger*

Various compounds were found to be produced in the presence of the fungal strain, which can be evaluated by observing the chromatogram (Fig. 4). The extensive compounds produced by *A. niger* it degraded wastewater included 2,4,7-Trinitrofluorenone, 8-Methyl-6-nonenamide, Benzothiophene-3(carboxylic acid), 4,5,6,7-tetrahydro-2-amino-6-ethyl-, Imidazole, 4,5-di(2-furyl)-2-(3-indolyl), Phenylacetamide, ethylester, N-ethyl-N-(3-methylphenyl)-, 12-Chloro-14-azatetracyclo[7.6.1.0(2,7).0(13,16)] hexadeca-1(15),2(7),3,5,9(16),10,12-heptaen-8-one, Methyl 3-amino-2-methylbenzoate, N, N-diacetyl, Benzonitrile, m-phenethyl, Benzothiophene-3-carboxylic acid, 4,5,6,7-tetrahydro-2-amino-6-ethyl-, ethyl ester, 2-Pentyl-6-phenyl-1H-pyrazolo[1,2-a] cinnoline-1,3(2H)-dione, 2,6-Lutidine 3,5-dichloro-4-dodecylthio, 4-(4-Acetamidophenyl)-2-aminothiazole, 2-[2-[2-(4-Chloro-phenoxy)-ethylsulfanyl]-benzoimidazol-1-yl]-acetamide, 3-Hydroxy-4'-methoxy-6-methylflavone, trifluoroacetate.

Table I. GC-MS analysis of compounds generated by *A. niger* during azo dye degradation.

Degradation products produce by <i>A. niger</i>	Activities reported	Reference
1. Tetradecane	Antibacterial, antifungal	Abd El-Rahim <i>et al.</i> (2021)
2. Eicosane	Anti-inflammatory, analgesic, and antipyretic effects	Costa <i>et al.</i> (2025)
3. Nonadecane	Antimicrobial, antioxidant	-
4. 3-Methyl-2,3,6,7,8,8a-hexahydropyrrolo[1,2-a]pyrazine-1,4-dione	Not reported	-
5. Azetidine, 1,1'-methylenebis	Not reported	-
6. Hexahydropyrrolizin-3-one	Not reported	-
7. L-Proline, N-(hexanoyl)-, pentyl ester	Not reported	-
8. 3-Cyclohexyl-L-alanine amide, N,N,N'-tetramethyl	Not reported	-
9. Octadecanoic acid	Antibacterial	Rodríguez <i>et al.</i> (1999)
10. Benzoic acid, 3,4,5-trihydroxy	Antioxidant, antimicrobial	Hadibarata <i>et al.</i> (2013)
11. Carbonic acid, monoamide, N-(2-pentyl)-N-propyl, allyl ester	Not reported	-
12. 6-Chloro-2-(4-nitro-phenoxy)-4-phenyl-quinazoline	Anticancer, antimicrobial	Syafiuddin and Fulazzaky (2021)
13. Pent-4-enoylamide,2-methyl-N-(2-butyl)-N-nonyl	Not reported	-
14. 1-Benzylindole,4,5-trihydroxy, carboxylic acid	Indole derivatives are often bioactive; this specific compound is not found	-
15. 9H-Fluoren-9-one	Antimicrobial	Ameen <i>et al.</i> (2021)
16. Ferrocene	Anticancer, antiproliferative, antimalarial (via derivatives)	Tang <i>et al.</i> (2019)
Degradation products produced by <i>P. simplicissimum</i>		
1. 5-Azacytosine, N,N,N'-trimethyl	Not reported	-
2. 5-Cyclohexyl-3H-1,3,4-oxadiazole-2-thione	Not reported	-
3. 1H-(2,1,3)-Benzothiadiazine, 3,4-dihydro-, 2,2-dioxide	Not reported	-
4. Undec-10-ynoylamide, N-(2-pentyl)-N-decyl	Not reported	-
5. Valeramide, N-(2-butyl)-N-nonyl	Not reported	-
6. Acetamide, 2-phenyl-N-(2-butyl)-N-nonyl	Not reported	-
7. 18-Nor-estra-1,3,5(10),9(11)-tetraen-12-one		-
Indole, 3-(4-nitrophenylamino)-	Indole derivatives possess antiviral, anti-inflammatory, anticancer, anti- HIV, antioxidant, antimicrobial.	Singh and Dwivedi (2020)
8. Quinoline, 6-[difluoro[6-(1-methyl-1H-pyrazol-4-yl)-1,2,4-triazolo[4,3-b]pyridazin-3-yl]methyl]-	Not reported	-
9. 1,4-Anthracenedione, 6-nitro	Not reported	-
10. 1H-Pyrrolo(2,3-b)pyridine, 3-amino-2-(4-pyridyl)-	Not reported	-
11. Benzene, 1-methoxy-4-(2-phenylethenyl)-	Not reported	-
12. 9H-Fluoren-9-one, 2,7-diamino	Not reported	-
13. 5-Phenylvaleric acid, 2-methyloct-5-yn-4-yl ester	Not reported	-
14. Pimelic acid, di(2-chlorophenyl) ester	Not reported	-

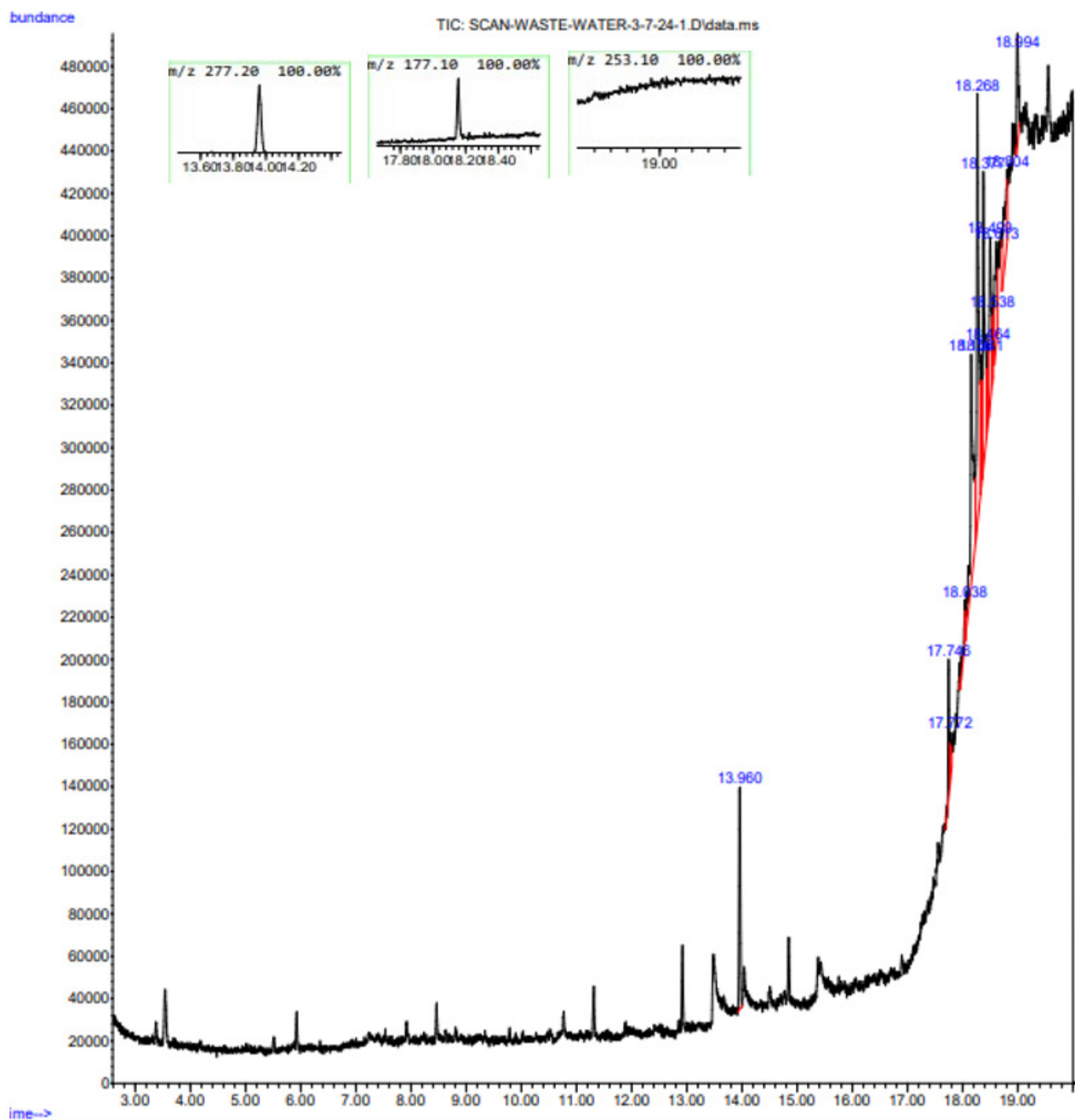


Fig. 4. Compounds produced in the presence of *A. niger* in wastewater containing Congo Red.

DISCUSSION

The wastewater from the textile industry contains many toxic and carcinogenic contaminants. Among them, the major contaminant is azo dye. The biodegradation of such harmful and carcinogenic contaminants is mediated by the utilization of microorganisms such as fungi, bacteria, and yeast, but the degradation of azo dyes using

fungal strains has been a topic of increasing interest in recent years because fungi are capable of breaking down complex organic pollutants into less toxic compounds such as phenolic intermediates. These intermediates can further be degraded, which results in the complete mineralization of azo dyes. The optimum conditions required for the degradation of dyes by fungi are pH 5-6, temperature 25-30°C, and the dye concentration 100-200 mg/L. However,

fungal growth and dye degradation potential can further be enhanced by the use of co-substrates, such as glucose and yeast extract.

Many fungal strains have been reported in the degradation of dyes due to their degradation capabilities (Abd El-Rahim *et al.*, 2021) because of the production of extracellular enzymes. It was reported that white rot fungi have been used for the breakdown of many dyes (Costa *et al.*, 2025). According to one study, white rot fungi have a degradation potential of about 52% because of the excretion of specific extracellular enzymes like Mn-peroxidase, laccase, etc. (Rodríguez *et al.*, 1999). *Pleurotus eryngii* F032 showed 93.56 % decolorization of Reactive Black 5 dye (Hadibarata *et al.*, 2013). Fungus strains *Trichoderma koningiopsis*, *Pestalotiopsis* sp., and *Trichoderma citrinoviride*, were responsible for degrading Remazol Brilliant Blue R by 52.5%, 74.8%, and 33.1%, respectively (Syafiuddin and Fulazzaky, 2021). According to the study conducted in 2023, *A. niger* gave 90%, 70%, and 40% decolorization on Navy-Blue, Synozol yellow, and red, whereas *Trichoderma viride* gave 87%, 73%, and 36%, respectively, for 60 days (Ali *et al.*, 2023).

In this study, *Aspergillus niger* and *Penicillium simplicissimum* were evaluated for their degradation potential. The maximum decolorization calculated by *A. niger* for Congo Red dye was 90% and by the *P. simplicissimum* was 91% for the same dye. Acid Blue and Disperse Red 1 dyes were also broken down by numerous *Aspergillus* strains (Ameen *et al.*, 2021).

In another study, the consortium of fungal and microalgae strains decolorized 98.09% of Disperse Red 3B dye (Tang *et al.*, 2019). Another study reported the successful degradation of azo dyes using a consortium of *Fusarium* sp. and *Trichoderma* sp. (Singh and Dwivedi, 2020). The use of consortia offers several benefits which including increased degradation efficiency, reduced toxicity, and improved adaptability to different environmental conditions (Kumar *et al.*, 2021). The GC-MS analysis of Congo Red dye-polluted water treated with *A. niger* generated several compounds, including benzene dicarboxylic acid and benzene propanoic acid as degraded products (Singh and Dwivedi, 2022).

According to the study conducted in 2023, *A. niger* and *T. viride*-treated wastewater containing Navy-blue, Synozol yellow, and Synozol red dyes produces many compounds such as caprolactam, oleic acid, and isopropyl benzene, etc., which are observed by GC-MS analysis (Ali *et al.*, 2023).

When *Aspergillus* sp. and *Chlorella sorokiniana* were combined, GC-MS analysis of a dye called Disperse Red produced a variety of compounds, including o-xylene, di-isobutyl phthalate, and acetone (Tang *et al.*, 2019). When *Trametes gibbosa* treated polluted Alizarin Red

wastewater, the compounds produced were analyzed and found to include 1-butylene and acrylaldehyde (Zhang *et al.*, 2021).

According to a recent investigation, the degraded products by *T. viride* and *A. niger* were found to be Arsenous acid, caprolactam, tris (trimethylsilyl) ester, Diglycolamine, and many others. According to the current investigation, the *A. niger* produced Tetradecane, Eicosane, Nonadecane, 3-Methyl-2,3,6,7,8,8a-hexahydropyrrolo[1,2-a] pyrazine-1,4-dione, Octadecanoic acid, Benzoic acid, 3,4,5-trihydroxy, 9H-fluoren-9-one, and many others on degrading Congo Red dye. Whereas, *P. simplicissimum* on degrading Congo Red dye resulted in the formation of Hexadecanoic acid, 1H-Pyrrolo[2,3-b] pyridine, 3-amino-2-(4-pyridyl)-, 1-methoxy-4-(2-phenylethenyl)-, 9H-fluoren-9-one, 2,7-diamino, (2,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, trans, 5-Phenylvaleric acid, 2-methyloct-5-yn-4-yl ester, Pimelic acid, di(2-chlorophenyl) ester.

Benzene, propanoic acid, and benzene dicarboxylic acid were the main byproducts of the degradation of Congo Red dye-polluted water treated with *A. niger* when it was examined through GC-MS (Singh and Dwivedi, 2022).

Moreover, in the current study, wastewater was treated with *A. niger*, which resulted in 70% decolorization, and GC-MS analysis of wastewater treated with *A. niger* produced 2,4,7-Trinitrofluorenone, 8-Methyl-6-nonenamide, Benzothiophene-3-carboxylic acid, 4,5,6,7-tetrahydro-2-amino-6-ethyl-, ethyl ester, Imidazole, 4,5-di(2-furyl)-2-(3-indolyl), Phenylacetamide and many others. Despite the promising results, there are some limitations associated with the fungal degradation of azo dyes. These include a slow rate of degradation, the formation of toxic intermediates, and the need for optimal conditions.

Overall, these findings suggest that fungal strains and consortia provide a promising solution for the degradation of azo dyes. Further research is needed to fully understand the mechanisms of fungal degradation and to optimize the process for industrial applications, thus providing a feasible solution to a toxicity-free environment.

CONCLUSION

This research work focused on the breakdown/degradation of azo dyes by fungal strains. Both fungal strains i.e., *A. niger* and *P. simplicissimum*, degraded azo dye upto 90% and 91%, respectively, and proved to be excellent bioremediators of contaminants, including azo dyes. During this degradation process, various compounds, including octadecanoic acid, benzoic acid, 3,4,5-trihydroxy, 9H-fluoren-9-one, and many others, were generated as byproducts. These dye-degradative compounds are useful as

they possess antioxidant and antimicrobial properties. The wastewater, after fungal treatment, can at least be used for crop irrigation to compensate for the scarcity of freshwater.

DECLARATION

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Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in the creation of this manuscript.

Statement of conflict of interest

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