EnvironmentAsia
The international journal by the Thai Society of Higher Education Institutes on Environment

EnvironmentAsia 16(2) 2023 66-75
DOI 10.14456/ea.2023.21
ISSN 1906-1714; ONLINE ISSN: 2586-8861

Investigation the Degradation Capabilities of Fungal Isolate from Water and Sediment Samples to Congo Red Dye

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Received: January 27, 2023; Revised: March 4, 2023; Accepted: March 31, 2023

Abstract

This study investigated fungal biodiversity in water and sediment samples and their ability to degrade Congo red (C.R.) in solid and liquid media. Ten fungal genera were isolated, and the genus *Aspergillus* represented the highest percentage of 70%, while the other genera ranged from 10% to 40%. Sixteen fungal species were isolated, and 88% of which (14 species) belonged to anamorphic fungi, 6% (1 species) to Zygomycota, and another 6% to Ascomycota. *Trichoderma* sp. showed the highest percentage of 40%. Out of the 16 isolated fungi, *Aspergillus niger*, *Trichoderma* sp. and *Penicillium* sp.1 showed the best result in decolourising C.R. on a solid potato dextrose agar medium (PDA). These fungi were selected to test their ability to biodegrade C.R. in a liquid medium (MSM) supplemented with 50 mg/L C.R. as the sole carbon source. After 7 days of incubation, *Trichoderma* sp. degraded 63% of C.R., *A. niger* degraded 33% and *Penicillium* sp.1 degraded 15%. Overall, *Trichoderma* sp. is a potential microorganism for C.R. degradation.

Keywords: Biodegradation; Congo red; Fungi; Water and sediments

1. Introduction

Fungi are the second largest biotic community after insects, with only 120,000 species of fungi identified so far, although the total number is estimated to be 2.2 – 3.8 million; as such, fungi are one of the biggest sources of biodiversity with fewer discoveries (Hawksworth and Lücking, 2017). The importance of fungi is not only due to their vital roles in ecosystem functions but also because of their effect on humans and human-related activities. Fungi have high biomass production and a large surface area and can easily adjust to adverse environments, such as those with different kinds of urban and industrial effluents and strongly contaminated by hydrocarbons, dyes, etc. (Chatterjee *et al.,* 2020; Buratti *et al.,* 2022).

The continuous growth of the population and increasing industrial activities in different sectors require the development of novel dyes with varying nature. Many kinds of synthetic dyes are produced annually by hundreds of thousands of tons all over the world (Varjani *et al.,* 2020; Selvaraj *et al.,* 2021). Among synthetic dyes used in different industries, azo dyes are the most commonly used, accounting for approximately 70%, due to their stability, chemical versatility, high fixation and resistance to light and moisture (Shi *et al.,* 2021). Azo dyes are chemically represented as (R-N = N-R'), with (-N = N-) being the chromophore group referred to as (Azo) connected to aromatic rings (Liu *et al.,* 2020). Congo red (C.R.) is a famous anionic diazo dye that is prepared by coupling tetrazotised benzidine with two molecules of napthienic acid; C.R. has been used extensively in textile, paper, rubber and plastic industries (Bouras *et al.,* 2017; Asses *et al.,* 2018). Disposal of untreated dyes attached with benzidine group poses major problems, such as tumorigenicity and carcinogenicity, due to their biotransformation to benzidine compound, representing

a great risk to humans and the environment (Guo *et al.,* 2020).

Decolourisation by fungi and yeasts have significant advantages, such as eco-friendliness, low processing cost, non-toxicity and complete mineralisation (Varjani *et al.,* 2020; Kamal *et al.,* 2022 Pinheiro *et al.,* 2022). Fungi can degrade azo dyes through synthesis of a number of ligninolytic enzymes, including laccase, lignin peroxidase and manganese peroxidase (Chatterjee *et al.,* 2020; Pinheiro *et al.,* 2022). Previous studies investigated C.R. degradation using fungi, such as *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus oryzae*, *Penicillium chrysogenum, Cladosporium rubrum and Pleurotus ostreatus* (Bhattacharya *et al.,* 2011; AI-Jawhari & Al-Mansor, 2017; Asses *et al.,* 2018; Kang *et al.,* 2018; Chatterjee *et al.,* 2020).

This research aimed to identify fungal diversity in water and sediment samples in Basrah province and evaluate their ability to degrade Congo red dye. It was the first work in Basrah to study the fungal diversity in the dyes contaminated area. Basrah suffered from intensive contamination by different contaminants including Azo dyes. The objective of this study was to use these fungi to biologically treat the contamination as ecofriendly method. This work will show the potential ability of fungi to degrade Congo red to clean up the contaminated area in Basrah province as a clean and ecofriendly technique. This is the first work in Basrah province which used fungi as remediation tool.

2. Materials and Methods

2.1 Sample area

Ten water and sediment samples were collected at 4 stations along Shatt Al-Arab River in Basrah province namely; Al-Hartha, Al-Karma, Al-Ashar and Hamdan. All of these stations receive variety of pollutants including Azo dyes from multiple human sources and in different quantities (Figure 1).

All samples were collected between October and November 2021. Approximately 150 – 200 g of sediment samples were collected and placed in clean bags, and water samples were taken by sterile bottles (Hashem *et al.,* 2018). All the samples were transferred to the laboratory and maintained at 4 °C until further use.

2.2 Chemicals and media

All the Chemicals, including C.R. and isolation media, were purchased from Hi Media (Mumbai, India) and Bioneer (Daejeon, South Korea).

2.3 Fungal isolation

Potato dextrose agar (PDA) and malt extract agar (MEA) were used for the primary isolation of fungi. The isolation media were prepared according to the protocols of the manufacturing

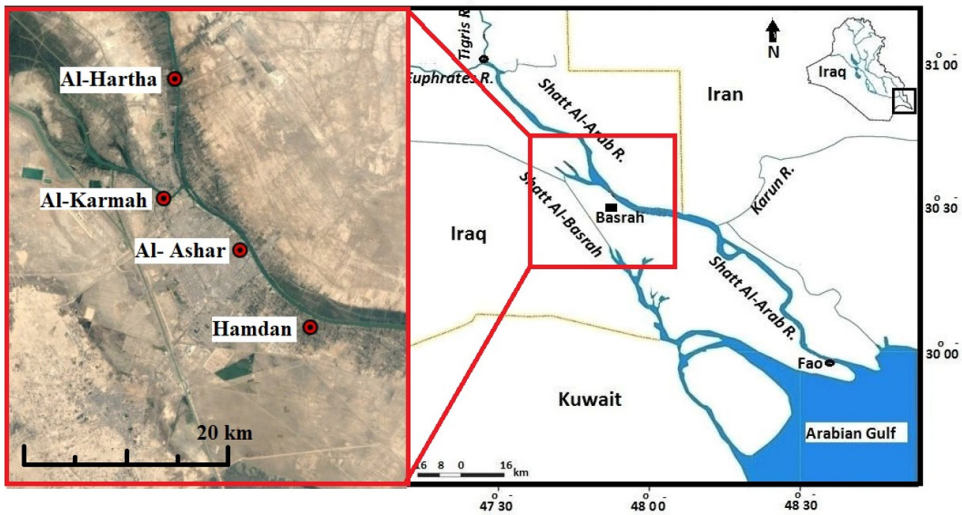


Figure 1. Study area and sampling stations

company (Hi media, India) and added with chloramphenicol (250 mg/L) to inhibit the growth of bacteria. Fungi were isolated from sediment samples by using the dilution method described by Wicklow and Wittingham (1974). A total of 10 g of soil was suspended in 90 mL of sterile distilled water to make a 10^{-1} dilution. For water samples, the dilution method of Khalid *et al.* (2016) was used, wherein 10 mL of each water sample was added to 90 mL of sterile distilled water to make a 10^{-1} dilution. After exhaustive shaking for 10 min, 1 mL of each dilution was transferred to a sterile Petri dish and added with 15 mL of sterile media. The components of the Petri dish were mixed well before solidification. All the dishes were incubated at 25 °C for 7 – 14 days. Pure cultures from all fungal colonies were maintained on PDA and MEA for morphological and molecular identification.

2.4 Identification of isolated fungi

The isolated fungi were identified phenotypically according to the following references: Raper and Fennell (1973), de Hoog and Guarro (1995), Watanabe (2010) and Guarro *et al.* (2012). Pure cultures from the isolated fungal colonies were subcultured on Petri dishes containing PDA medium and allowed to grow for 7 days for molecular identification. DNA extraction and PCR amplification were performed as described by Mirhendi *et al.* (2006). A forward primer ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and a reverse primer ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) (19 and 20 base pairs [bp] long, respectively) were used for the isolates. The purification and sequencing of the PCR products for the recovery of isolates was carried out at Macrogen (Seoul, South Korea). The obtained sequences were corrected and compared with nucleotide sequences of NCBI using basic local alignment search tool (BLAST). The percentages of appearance for the fungal isolation were calculated according to the following equation;

$$\% \text{ Percentage of appearance} = \frac{\text{No.of samples in which the genus or species appeared}}{\text{The total No.of samples}} \times 100\%$$

2.5 Preliminary screening for fungal isolates to degrade C.R. on solid medium

The ability of the isolated fungal species to grow and degrade C.R. on a solid medium was studied according to Sumathy (2014). C.R. solution (50 mg/L) was added to the PDA medium after autoclaving at 15 lb/inch^2 for 20 min and allowed to solidify. From each fungal isolate, a mycelium disk was made by a cork borer (5 mm) and inoculated into the PDA medium containing C.R. All the dishes were incubated at 25 °C for 7 days. Two types of controls were prepared. The first control containing the medium and the fungal isolate without C.R. was used to compare the fungal growth with and without C.R. and calculate the percentage of inhibition. The second control containing C.R. without any fungal isolate was used to compare the observable fading of C.R. colour from the inoculated plate.

Mycelium diameter growth and colour intensity change were measured at 72 h intervals. The percentage of inhibition of fungal growth during degradation was measured and calculated using the following formula;

$$I = C\text{-}T/C \times 100$$

Where; I is the percentage inhibition in fungal growth, C is the growth in terms of colony diameter in control and T is the growth in terms of colony diameter in the sample.

2.6 Biodegradation of Congo red in liquid medium

The fungal isolates that lodged the best results in the prior experiment were further tested to evaluate their capability to degrade C.R. in liquid medium. The isolates were activated by subculturing it on PDA medium for 7 days. The liquid medium used was mineral salt medium (MSM, g/L) containing 0.75 g of KH_2PO_4 , 0.75 g of K_2HPO_4, 0.05 g of $MgSO_4.7H_2O$, 0.05 g of $CaCl_2.2H_2O$, 0.02 g of $FeSO_4.7H_2O$ and 0.1 g of yeast extract per litre of distilled water (AI-Jawhari and AL-Mansor, 2017).

Conical flasks (250 mL) containing 100 mL of MSM (pH 6) supplemented with 50 mg/L Congo red as the sole carbon source were inoculated with three plugs of mycelia (5 mm diameter) obtained from the edge of actively growing mycelia. The culture flasks were incubated at 25 °C for 7 days. Control flasks consisting of media with dye only were also prepared. All experiments were carried out in triplicate. After 7 days, a 10 mL aliquot was taken from all culture flasks including the control and centrifuged for 10 min (5000 rpm). The maximum absorbance at 498 nm was recorded using UV/VIS spectrophotometer to determine the concentrations of C.R. dye. The biodegradation of C.R. was then calculated using the following formula;

$$\%\text{Degradation} = \frac{\text{initial absorbance} - \text{observed absorbance}}{\text{initial absorbance}} \times 100\%$$

Where; initial absorbance is the control and the observed absorbance is the treatment absorbance (Hashem *et al.,* 2018).

2.7 Statistical analysis

All the data were expressed as mean ± SD. One-way ANOVA was conducted using Minitab software (version 20) to determine significant differences among fungal samples.

3. Results and Discussion

3.1 Identification of isolated fungi

3.1.1 Identification of fungal genera

In this study, 10 fungal genera and sterile mycelia were isolated from 10 water and sediment samples (Table 1). About 88% of the isolated fungi were anamorphic fungi belonging to 14 species. The high percentage of anamorphic fungi might be due to the capability of these fungi to produce a vast number of reproductive units that support their widespread distribution in the environment. These fungi can also tolerate adverse environmental conditions and secrete several enzymes, which help them degrade a broad range of organic compounds. In this regard, anamorphic fungi are one of the primary groups of fungi in the environment (Ziaee *et al.,* 2018; Altaee and Al-Dossary, 2021). Two phyla, namely, Zygomycota and Ascomycota, appeared in a low percentage (6% for each one) possibly because they cannot grow under extreme conditions and high temperatures; they also need specific isolation methods and special culture media for isolation or may grow slowly, especially Ascomycota, requiring longer growth periods (Raja *et al.,* 2017; Wu *et al.,* 2019). The percentage of fungal genera ranged from 10% to 70 %, and *Aspergillus* had the highest percentage of 70%. This genus can withstand and adapt to difficult conditions including a wide range of temperatures and can release enzymes, such as lignin and manganese peroxides, which help them degrade organic substances as sources of energy and growth; these fungi also have many stress-tolerant asexual conidia (Tian *et al.,* 2017; Ziaee *et al.,* 2018). Other genera isolated ranged from 10% to 40%, and *Trichoderma* recorded the second highest percentage of 40%. This genus; a type of anamorphic fungi, similar to *Aspergillus*, also produce a huge number of conidia and can adapt to different types of environments (Tian *et al.,* 2017). The appearance of some genera in low percentage does not mean that they do not play an important environmental role and may be effective in their original environment. The method of isolation, the duration of incubation and the isolation media used in the study may be inappropriate for their growth; they may also grow slowly and need longer time to grow up or cannot compete with other fungi. Differences in fungal appearance also depend on season and temperature (Saramanda and Kaparapu, 2017; Zhang *et al.,* 2020). The results of the current study are consistent with those reported by Abdullah *et al.* (2010), Al-Saadoon and Al-Dossary (2010 & 2014), Al-Daamy *et al.* (2018), Minati *et al.* (2020) and Lima *et al.* (2021); that is, the genus *Aspergillus* recorded the highest percentage.

3.1.2 Identification of fungal species

Sixteen fungal species were isolated from 10 water and sediment samples (Table 2). The percentage of these species ranged from 10% to 40%. *Trichoderma* sp. showed the highest percentage (40%) and appeared in four different samples. This species has enzymatic capabilities that enable it to degrade a variety of substances in the environment, including toxic substances, and has high ability to adapt and live in various environmental conditions; it also plays an important environmental role because of its strong competition with other microorganisms in the environment and exhibits hyperparasitism, which enables it to grow and spread (He *et al.,* 2018; Singh *et al.,* 2018). *A. flavus* and *A. niger* had the second highest percentage of 20%, and the rest of the species, such as *Acremonium* sp. appeared in a low percentage of 10% (Table 2). In general, the numbers of species isolated were lower than those in other studies. Wu *et al.* (2022) isolated 299 fungal species from 33 different sediment samples. The difference may be due to the number of samples collected, the nature of the environment from which the fungi where isolated, the sample collection period and the high temperatures during the sample collection because it may adversely affect the growth of microorganisms and reduce their numbers; all these factors affect the fungal diversity (Raja *et al.,* 2017; Abood, 2018).

Table 1. The isolated fungal genera with their percentage of occurrence

No.	Fungal genera	No. of samples in which the genus appeared	% of appearance
1	*Acremonium*	1	10
2	*Aspergillus*	7	70
3	*Cladosporium*	1	10
4	*Ectophoma*	1	10
5	*Monascus*	1	10
6	*Mucor*	1	10
7	*Penicillium*	3	30
8	*Phialophora*	1	10
9	*Phoma*	1	10
10	*Trichoderma*	4	40
11	Sterile mycelia	3	30

Table 2. The isolated fungal species with their percentage of appearance

No.	Fungal species	No. of samples in which the species appeared	% of appearance
1	*Acremonium* sp.	1	10
2	*Aspergillus allahabadii*	1	10
3	*A. flavus*	2	20
4	*A. fumigatus*	1	10
5	*A. niger*	2	20
6	*A. terreus*	1	10
7	*Cladosporium* sp.	1	10
8	*Ectophoma multirostrata*	1	10
9	*Monascus pallens*	1	10
10	*Mucor* sp.	1	10
11	*Penicillium* sp.1	1	10
12	*Penicillium* sp.2	1	10
13	*Penicillium* sp.3	1	10
14	*Phialophora* sp.	1	10
15	*Phoma* sp.	1	10
16	*Trichoderma* sp.	4	40

3.2 Decolourisation of C.R. on solid medium

The ability of the isolated fungi to decolorize C.R. was tested on PDA medium. The degradation efficiency of the tested fungi was determined based on their growth, inhibition percentage and decolourisation ability (Table 3).

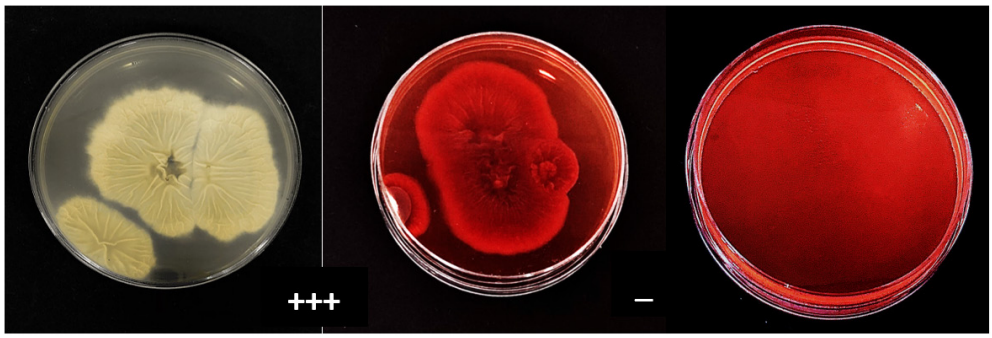
The growth of most fungi on the medium was affected by the C.R. dye but in varying degrees. The inhibition percentage ranged from 6% to 41%. *A. fumigatus* was significantly affected by C.R. in the medium and showed maximum inhibition (41%), while the minimum inhibition was found in *Penicillium* sp.1 (6%) (Table 3). Some fungi were sensitive toward the toxic effect of C.R., while other fungi did not show a considerable effect, indicating that they can adapt to toxic compounds in the environment; they can tolerate the toxic effects of the dye, degrade it and use it to grow (Purnomo *et al.,* 2019). In a similar study, Vasdev (2011) stated that the growth of six species of white-rot fungi was not affected by the existence of dyes in the medium, assuming that some fungi can tolerate and decolorize dyes for growth.

Of the 16 tested fungi, 11 were found to have the ability to decolorize C.R. on solid medium. *A. niger* and *Trichoderma* sp. had the best efficiency of decolourisation (+++), with visible disappearance of C.R. colour, compared with the control. These fungi have unique enzyme systems for degrading complex organic compounds (Table 3, Figure 2) (Singh and Singh 2010). The ability of the remaining fungi to decolorize C.R. ranged from (—) with no colour changing, such as in the case of *Phialophora* sp., to (++) with good decolourisation efficiency, such as in the case of *A. flavus*. Visual measuring for the color change was supported by a number of researchers and the preliminary method to see the ability of different fungi to degrade Congo red. In this method if there was slightly change in the color from dark red to colorless it take (+), and so on if medium color changed completely from dark red to colorless it take (+++).The results are consistent with the reports of Singh and Singh (2010), AI-Jawhari and AL-Mansor (2017), Bosco *et al.* (2017) and Chatterjee *et al.* (2020), who discovered that *Trichoderma harzianum, Penicillium funiculosum, Phanerochaete chrysosporium* and *A. flavus* can decolorize C.R. on solid medium. Jayasinghe *et al.* (2008) stated that some fungi, such as *Pycnoporus coccineus, Fomes fomentarius, Stereum ostrea* and *Pycnoporus cinnabarinus* have good mycelium growth in a medium containing the dye, but they could not decolorize it.

Table 3. Growth of fungal mycelium in solid medium

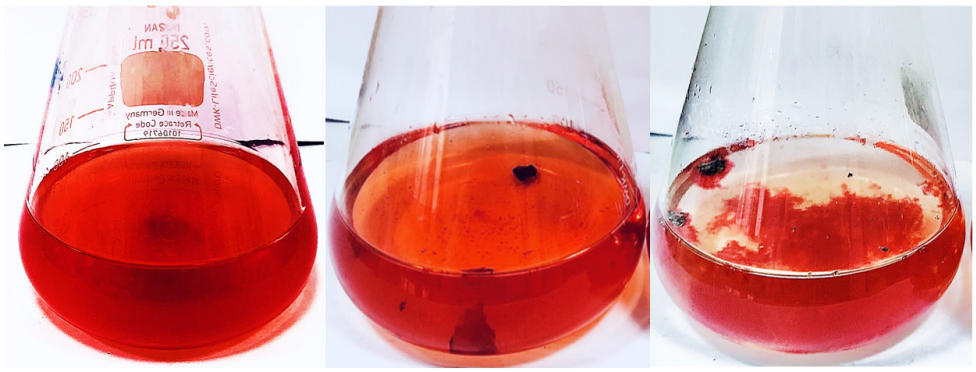
Fungi	Fungal growth in Control (in cm)	Fungal growth in Sample (in cm)	Inhibition%	Decolourisation degree
Acremonium sp.	6 ± 0.28	5 ± 1.91	24	-
Aspergillus allahabadii	6 ± 1.06	5 ± 0.35	14	+
A. flavus	6 ± 0.70	5 ± 0.35	21	++
A. fumigatus	8 ± 0.99	5 ± 2.40	41	+
A. niger	8 ± 0.14	6 ± 2.82	25	+++
A. terreus	8 ± 0.14	6 ± 0.07	16	+
Cladosporium sp.	8 ± 1.41	6 ± 3.39	23	-
Ectophoma multirostrata	7 ± 0.56	6 ± 1.20	21	+
Monascus pallens	6 ± 0.14	3 ± 0.14	18	+
Mucor sp.	8 ± 0.35	5 ± 2.47	30	-
Penicillium sp.1	8 ± 1.06	8 ± 0.42	6	++
Penicillium sp.2	8 ± 0.99	7 ± 2.83	28	++
Penicillium sp.3	8 ± 1.06	6 ± 1.63	29	+
Phialophora sp.	7 ± 1.06	7 ± 0.70	13	-
Phoma sp.	8 ± 0.70	6 ± 2.61	30	-
Trichoderma sp.	9 ± 0.00	7 ± 0.42	19	+++

Abbreviations: − no color change; + low color change; ++ mild color change; +++ high color change.



A. niger *Phialophora* sp. Control

Figure 2. Visual decolourisation of Congo red in solid medium



Control *Penicillium* sp.1 *Trichoderma* sp.

Figure 3. Visual decolourisation of Congo red in liquid medium

3.3 Decolourisation of C.R. in liquid medium

Three fungal isolates, namely, *A. niger*, *Trichoderma* sp. and *Penicillium* sp.1, which showed the best result in the decolourisation of C.R. on the solid medium, were selected to study their ability to degrade C.R. in a liquid medium. *Trichoderma* sp. showed the highest degradation percentage of 63% for C.R. within 7 days (Figure 3) possibly because of their high ability to tolerate the toxicity of the dye and grow faster than the two other species; they also have extracellular enzymes, such as laccase, which is involved in C.R. degradation (Balcázar-López *et al.,* 2016; Bagewadi *et al.,* 2017). The result is consistent with the study of Bagewadi *et al.* (2017), who asserted that *T. harzianum* can degrade 60% of the C.R. dye.

A. niger degraded 41% of the dye. C.R. may be so toxic for this species or it may need more time to grow and degrade the dye. The result is consistent with the research of Asses *et al.* (2018) and Liu *et al.* (2020), who reported that *A. niger* can degrade 45% of the C.R. dye and *Aspergillus* sp. can degrade 45.5%, respectively.

Penicillium sp.1 showed the lowest degradation efficiency of 15% (Figure 3), which may be due to the low ability of its enzymes to degrade C.R. or the high toxicity of the dye. This result is consistent with the study of Bhattacharya *et al.* (2011), who found that P. *chrysogenum* degraded 15% of the Congo red dye.

4. Conclusion

This study, 16 fungal species were isolated and their ability to degrade C.R. was investigated. *Aspergillus* had the highest percentage of 70%, followed by *Trichoderma* sp. (40%). Out of 16 fungal isolates, three fungal species, namely, *A. niger*, *Trichoderma* sp. and *Penicillium* sp.1

showed the best result for decolourisation of C.R. on the solid medium and were selected for testing on liquid medium. *Trichoderma* sp. showed the best result to degrade C.R. in the liquid medium with dye as the sole carbon source, where 63% of the dye was degraded after 7 days of incubation. As a conclusion, fungi was used as bioremediation agents to treat contaminated water before it is released into the environment or incorporated into constructed wetlands to work with plants in the remediation of contaminated water.

References

Abdullah SK, Al-Dossari MN, Al-Imara FJ. 2010. Mycobiota of surface sediments in marshes of Southern Iraq. Marsh Bulletin 2010; 5(1): 14-26.

Abood SA. Effect of biostimulation factors and bioaugmentation on crude oil degradation by using some species of fungi and bacteria isolated from some polluted soils in Basrah governorate *In- vetro*. M.Sc. thesis University of Basrah, College of Science 2018; 152 pp.

Al-Daamy AAH, Ahmed A, Mohammad G. Antimicrobial agents production by fungi isolates from the whisperers. Scientific Journal of Medical Research 2018; 2(06): 104-107.

AI-Jawhari IF, Al-Mansor KJ. Biological removal of malachite green and congo red by some filamentous Fungi. International Journal of Environment, Agriculture and Biotechnology 2017; 2(2): 238723.

Al-Saadoon AH, Al-Dossary MAN. Some fungi isolated from submerged plant debris in southern Iraq. Marsh Bulletin 2010; 5: 207-221.

Al-Saadoon AH, Al-Dossary MN. Fungi from submerged plant debris in aquatic habitats in Iraq. International Journal of Biodiversity and Conservation 2014; 6(6): 468-487.

Altaee MS, Al-Dossary MA. Evaluation of the enzymatic activity of some fungi isolated from plastic contaminated soils and their LDPE biodegradation ability. Marsh Bulletin 2021;16(2).

Asses N, Ayed L, Hkiri N, Hamdi M. Congo Red Decolorization and Detoxification by *Aspergillus niger*: Removal Mechanisms and Dye Degradation Pathway. Bio Med Research International 2018; 1–9.

Bagewadi ZK, Mulla SI, Ninnekar HZ. Purification and immobilization of laccase from *Trichoderma harzianum* strain HZN10 and its application in dye decolorization. Journal, genetic engineering & biotechnology 2017;15(1): 139–150.

Balcázar-López E, Méndez-Lorenzo LH, Batista-García RA, Esquivel-Naranjo U, Ayala M, Kumar VV, Savary O, Cabana H, Herrera-Estrella A, Folch-Mallol JL. Xenobiotic Compounds Degradation by Heterologous Expression of a Trametes sanguineus Laccase in *Trichoderma atroviride*. PLoS ONE. 2016; 11(2).

Bhattacharya S, Das A, Mangai G, Vignesh K, Sangeetha J. Mycoremediation of Congo red dye by filamentous fungi. Brazilian Journal of Microbiology 2011; 42(4): 1526–1536.

Bosco F, Mollea C, Ruggeri B. Decolorization of Congo Red by *Phanerochaete chrysosporium*: the role of biosorption and biodegradation. Environmental Technology 2017; 38(20): 2581-2588.

Bouras HD, Yeddou AR, Bouras N, Hellel D, Holtz MD, Sabaou N, Chergui A, Nadjemi B. Biosorption of Congo red dye by Aspergillus carbonarius M333 and Penicillium glabrum Pg1: Kinetics, equilibrium and thermodynamic studies. Journal of the Taiwan Institute of Chemical Engineers. 2017; 80:915-23.

Buratti S, Girometta CE, Baiguera RM, Barucco B, Bernardi M, De Girolamo G, Malgaretti M, Oliva D, Picco AM, Savino E. Fungal diversity in two wastewater teatment plants in North Italy. Microorganisms 2022; 10: 1096.

Chatterjee S, Dey S, Sarma M, Chaudhuri P, Das S. Biodegradation of Congo Red by manglicolous filamentous fungus *Aspergillus flavus* JKSC-7 isolated from Indian Sundabaran Mangrove Ecosystem. Applied Biochemistry and Microbiology 2020; 56(6): 708–717.

De Hoog GS, Guarro J. Atlas of clinical fungi. CBS Netherland and universitatRoviraVirgili. Spain. 1995; 720.

Guarro J, Gene J, Stachigel AM, Figueras J. Atlas of soil Ascomycetes. CBS-KNAW fungal biodiversity center Utrecht 2012; 486.

Guo G, Hao J, Tian F, Liu C, Ding K, Zhang C, Yang F, Xu J. Decolorization of metanil yellow G by a halophilic alkalithermophilic bacterial consortium. Bioresource Technology 2020; 316:123923.

Hashem RA, Samir R, Essam TM, Ali AE, Amin MA. Optimization and enhancement of textile reactive Remazol black B decolorization and detoxification by environmentally isolated pH tolerant *Pseudomonas aeruginosa* KY284155. AMB Express 2018;8: 83.

Hawksworth DL, Lücking R. Fungal diversity revisited: 2.2 to 3.8 million species. Microbiol Spectrum 2017; 5(4).

He XL, Song C, Li YY, Wang N, Xu L, Han X, Wei DS. Efficient degradation of azo dyes by a newly isolated fungus *Trichoderma tomentosum* under non-sterile conditions. Ecotoxicology and Environment Safety 2018; 150: 232–239.

Jayasinghe C, Imtiaj A, Lee GW, Im KH, Hur H, Yang HS, Lee TS. Degradation of three aromatic dyes by white rot fungi and the production of ligninolytic enzymes. Mycobiology 2008; 36:114–120.

Kamal IM, Abdeltawab NF, Ragab YM, Farag MA, Ramadan MA, Biodegradation,decolorization, and detoxification of Di-Azo dye direct red 81 by halotolerant, alkali-thermo-tolerant bacterial mixed cultures. Microorganisms 2022; 10:994.

Kang Y, Xu X, Pan H, Tian J, Tang W and Liu S. Decolorization of mordant yellow 1 using *Aspergillus* sp. TS-A CGMCC 12964 by biosorption and biodegradation. Bioengineered 2018; 9: 222–232.

Khalid T, Fatima A, Shafiq A, Javed S, Nadeem SG. Microbial decolorization of textile effluent. RADS J Biological Research and Applied Sciences 2016; 28–34.

Liu S, Xu X, Kang Y, Xiao Y, Liu H. Degradation and detoxification of azo dyes with recombinant ligninolytic enzymes from *Aspergillus* sp. with secretory overexpression in Pichia pastoris. Royal Society Open Science 2020; 7: 200688.

Liu X, Li W, Hu R, Wei Y, Yun W, Nian P, Feng J, Zhang A. Synergistic degradation of acid orange 7 dye by using non-thermal plasma and g-C3N4/TiO2: performance, degradation pathways and catalytic mechanism. Chemosphere 2020; 249:126093.

Lima LM, Okamoto DN, Passarini MR, Gonçalves SS, Goldman GH, Silveira MA, Ramos PL, Cruz JB, Juliano M, Marcondes MFM, Vasconcellos S. Enzymatic diversity of filamentous fungi isolated from forest soil incremented by sugar cane solid waste. Environmental Technology 2021; 3:1-10.

Minati MH, Mohammed-Ameen MK. Fungal diversity of winter wheat parts, seed and field soil in Iraq, Basra Province. Materials Science and Engineering 2020; 928(6):1-18.

Mirhendi H, Makiumura K, Khoramizadeh M, Yamagushi H. A one-enzyme PCR-RFLP assay for identification of six medically important *Candida* species. Japanese Journal of Medical Mycology 2006; 47: 225–229.

Raja HA, Miller AN, Pearce CJ, Oberlies NH. Fungal identification using molecular tools: a primer for the natural products research community. Journal of Natural Products 2017; 80(3): 756–770.

Raper K, Fennell DI. The genus *Aspergillus*. Sec. ed. Robert Krieger Publ. New York. 1973; 686.

Pinheiro LRS, Gradíssimo DG, Xavier LP, Santos AV. Degradation of azo dyes: bacterial potential for bioremediation. Sustainability 2022; 14(3): 1510.

Purnomo AS, Mauliddawati VT, Khoirudin M, Yonda AF, Nawfa R, Putra SR. Bio-decolorization and novel bio-transformation of methyl orange by brown-rot fungi. International Journal of Environmental Science and Technology 2019; 16: 7555–7564.

Saramanda G, Kaparapu J. Impact of pesticides on selected soil mycoflora. International Journal of Advanced Research and Biological Sciences 2017; 4(1): 105-112.

Selvaraj V, Swarna KT, Mansiya C, Alagar M. An over review on recently developed techniques, mechanisms and intermediate involved in the advanced azo dye degradation for industrial applications. Journal of Molecular Structure. 2021; 1224: 129-195.

Shi Y, Yang Z, Xing L, Zhou J, Ren J, Ming L, Hua Z, Li X, Zhang D. Ethanol as an efficient cosubstrate for the biodegradation of azo dyes by *Providencia rettgeri*: Mechanistic analysis based on kinetics, pathways and genomics. Bioresource technology 2021; 319: 124117.

Singh A, Shukla N, Kabadwal B, Tewari A, Kumar J. Review on Plant-*Trichoderma*-Pathogen Interaction. International Journal of Current Microbiology and Applied Sciences 2018; 7: 2382–2397.

Singh L, Singh VP. Microbial degradation and decolourization of dyes in semi-solid medium by the fungus – *Trichoderma harzianum*. International Journal of Science and Technology 2010.

Sumathy J. Biodegradation of textile azo dyes using fungi. Postgraduate & Research Department of Biotechnology, Women's Christian College, Chennai–600006, India 2014; 2: 611-621.

Tian X, Yang T, He J, Chu Q, Jia X, Huang J. Fungal community and cellulose-degrading genes in the composting process of Chinese medicinal herbal residues. Bioresource and Technology 2017; 241: 374-383.

Vasdev K. Decolorization of triphenylmethane dyes by six white-rot fungi isolated from nature. Journal of Bioremediation and Biodegradation 2011; 2(5):61–66.

Varjani S, Rakholiya P, Yong Ng H, You S, Teixeira JA. Microbial Degradation of Dyes: An overview, Bioresource Technology. 2020.

Watanabe T. Pictorial atlas of soil and seed fungi morphologies of cultured fungi and key to species, Third Edition. Boca Raton: CRC Press. 2010; 1: 426.

Wicklow DT, Wittingham C. Soil micro fungal changes among the profiles of disturbed conifer hard wood forest. Ecology. 1974; 55: 3-16.

Wu B, Hussain M, Zhang WW, Stadler M. Current insights into fungal species diversity and perspective on naming the environmental DNA sequences of fungi. An International Journal on Fungal Biology 2019; 10(3):127–140.

Wu KY, Liu YC, Mo L, Sun ZW, Liu ZY, Chen ZH, Huang RM, Zhang X. Effects of environmental factors on fungal diversity and composition in coastal sediments from Guangdong, China. SSRN Electronic Journal. 2022;1-38.

Zhang S, Fan F, Meng F. Seasonality and community separation of fungi in a municipal wastewater treatment plant. Applied and Environmental Microbiology 2020; 86(18): e00191-20.

Ziaee A, Zia M, Goli M. Identification of saprophytic and allergenic fungi in indoor and outdoor environments. Environmental Monitoring and Assessment 2018; 190: 574.