



## Research article

# Screening and identification of microbes from polluted environment for azodye (Turquoise blue) decolorization

Birhanu Gizaw<sup>\*</sup>, Tesfaye Alemu, Girma Ebsa

Department of Microbial, Cellular and Molecular Biology, Addis Ababa University, Addis Ababa, P. O. Box: 1176, Ethiopia



## ARTICLE INFO

## Keywords:

Azodye  
Biolog  
Biodecolorization  
MALDI-TOF  
Mycoremediation

## ABSTRACT

Turquoise blue dye is frequently used for industrial dyeing applications. But the release of untreated colored wastewater became an environmental and public health hazard. Microbial remediation of Azodye is environmentally safe and an alternative to a physicochemical approach. The aim of this research is to isolate and characterize turquoise blue dye degrading microbes from polluted environment. Microbial isolation and purification from soil and effluent sample was done on PDA and NA. Turquoise blue dye degrading test was investigated under optimized conditions using -the definitive screening design method. UV-Vis spectrophotometer used to measure the degradation percentage at 620 nm and 25 °C. The results revealed that 24 fungi and 6 bacterial species were identified from the contaminated site using Biolog Microstation and MALDI-TOF. Among all identified microbial species *Penicillium citrinum* Thom BCA & *Penicillium heriquei* show the highest percentage decolorization of turquoise blue dye up to 300 ppm with 90 % removal at pH4 and 87 % at pH 7 up to 400 ppm respectively. The azodye degradation ability of these fungi species used in the development of mycoremediation technologies provide an alternative option for Azodye removal after HPLC analysis, molecular characterization, and toxic analysis.

## 1. Introduction

Industrial development and rapid population growth have a direct impact on environmental safety. The growth of the global population demanding high quality textile products, thus Azodye containing wastewater, became a significant threat to the environment and water quality [1]. In Ethiopia, the textile sector is the third-largest and fastest-growing manufacturing industry and one of the primary top list source of water pollution [2,3]. According to Ref. [4] research conducted at the Bahir Dar textile industry in Amhara regional state, the liquid waste released from the factory causes significant harm to the aquatic ecosystem and disturbs the downstream users of the Blue Nile River [5]. studied the environmental and health consequences of effluents from textile and garment facilities in Gelan and Dukem of Oromia regional states, revealing that factories are causing hazards that exceed the federal environmental protection agency's allowed limit (FEPA) for residents and the surrounding environment [6]. Conducted an evaluation of industrial effluent pollution on the Borkena River in Kombolcha Textile, Ethiopia. Pollution levels were over the permitted limits of ambient surface water quality set by the EEPA at the downstream site of the Borkena River (see Table 6).

Synthetic Azo dye was widely used in various industries such as textile, printing, paper, tannery, pharmaceutical, food, cosmetics and manufacturing, accounting for more than 70 % of total dye usage with a global production of 1,000,000 tons [7–10]. During the

<sup>\*</sup> Corresponding author.

E-mail address: [gizachewbirhan@gmail.com](mailto:gizachewbirhan@gmail.com) (B. Gizaw).

dyeing process, up to 10–25 % of textile dyes are discharged into the environment without adequate treatment. The waste-containing dyes can remain in the environment for a long period of time [10–16]. In addition, untreated textile sludge may pose threats to human and animal health due to the leaching of hazardous heavy metals in agricultural lands, which has an impact on the quality and safety of food. For instance, most industries in Ethiopia dispose of untreated sludge in unsafe areas and open landfills, with approximately 30,000 tons annually from all industry parks [17]. At extremely low quantities of Azo dyes, about 10–50 mg/L, even for some dyes less than 1 ppm are water soluble and -cause visible color change in the water body. This has become a challenge for aquatic fauna and flora due to the reduction of light penetration and inhibition of gas dissolution [18–21]. In Ethiopia, about 15million kg of chemicals and dyestuff are used, and the majority are reactive dye and among this Turquoise blue dye covers a large portion. It is a vibrant versatile colorant that offers excellent color fastness and compatibility with various substrates due to its covalent bond [22]. It is easily applied to a wide range of materials, including cotton; silk, as well as synthetic fibers like polyester and nylon. The dye can also be used on non-textile substrates such as plastics and ceramics, making it more popular for various fields of applications. About 95 % of reactive dyes are azo dyes covering an entire range of colors. In the Color Index (CI) system, Azo dyes are grouped with numbers ranging from 11,000 to 39,999 in correspondence with the chemical structure, particularly Turquoise Blue (#4DD0E1)- RGB 77, 208, 225 has color information [23]. Due to one or more Azo (-N = N-) bonds connected to benzene or naphthalene rings and linking with aromatic amines, that Azo dyes are difficult to break and resist cleavage [24]. 50 % of textile effluent has residual Azo dye, which is very difficult to remove by conventional physical and chemical treatments [25]. In addition to that, physical and chemical treatment methods have some disadvantages, such as high cost, toxic by-product residue formation, excessive energy consumption, and concentrated sludge formation [26]. Therefore, biological treatment techniques using microbes are an alternative good option [27]. Because of their adsorbent properties, extracellular enzyme synthesis, their application's in economic viability and environmental friendliness, both live and dead fungal biomass has been employed currently to remove dyes including *Aspergillus* sp., *Rhizopus* sp., *Candida* sp., *Pleurotussp.*, *verticillum* and *Sacchromyces* sp., *Tichoderma* sp., *Penicillium* sp., *Geotricum candidum*, *Phanerochaete chrysosporium* etc [28–32]. This study aims to isolate and characterize microbes from polluted sites and select potential Azodye (turquoise blue) degrader fungi under optimized condition for bioinoculant formulation for bioremediation purposes.

## 2. Material and methods

### 2.1. Study area description and sample collection method

The study was conducted in selected urban agriculture site and rivers in Addis Ababa (9° 01' 29" N & 38° 44' 48" E, altitude 2355 m. a.s.l) & central rift valley of Ethiopia particularly from, Zeway, koka, Arbaminch and Mojo (approximately between 38°15'E and 39°20'E, and 7°10'N to 8°30'N, altitude 1600 m.a.s.l). Currently, In Addis Ababa 106,280 urban vegetable producers are registered in different site, and more than 300 ha who supply with rough estimates about 60 % of the city's leafy vegetables consumption [33]. The reason both study area were selected, that urban vegetable farming activities were high and irrigated by a river that was connected to textile and other several industry effluents and the pollution was high due to different industries. Regarding sample collection, 10 g of vegetable rhizosphere soil from farm site and 10 mL of wastewater samples from effluent and polluted rivers were collected and stored in a sanitized falcon tube (45 mL). Totally 150 falcon tube samples were gathered. The collected samples were transported to the Addis Ababa University mycology laboratory.

### 2.2. Equipment, reagent and chemicals

The chemicals and solvents used in the experiment were all of analytical grade. Biolog Universal Agar (BUY), Potato dextrose agar (PDA), and Nutrient agar (NA), were obtained from Sigma Aldrich Company, Biolog microstation, Turbidimeter (Biolog Inc., Hayward, USA) product, MALDI-TOF (Bruker MALDI Biotyper Microflex LT, Bruker Daltonics, Bremen, Germany, Jenway 6405 Uv-Vis spectrophotometer (U.K, jenway Ltd).

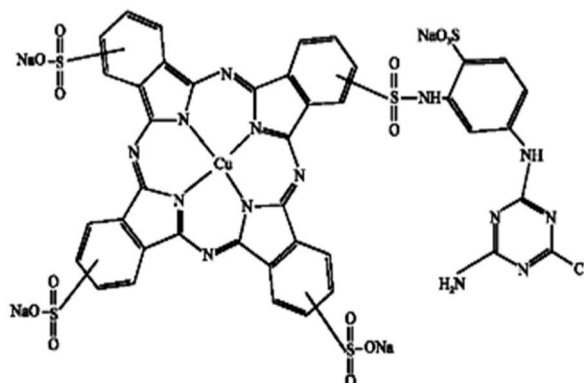


Fig. 1. Turquoise blue dye chemical structure [34].

### 2.3. The dye

The turquoise blue textile dye (99 % purity) used in this study was obtained from Sigma Chemical Company. This dye has the molecular formula  $C_{40}H_{25}CuN_9O_{14}S_5$  with a molecular structure, as shown in Fig. 1.

### 2.4. Microbial isolation and purification

Fifteen composite soil samples were pooled from 150 samples. Then 10 mL of waste water and 10 g of soil samples were transferred into 90 mL distilled water for each pooled composite sample. Serial dilution techniques were conducted from  $10^{-1}$  to  $10^{-6}$  mL. 0.1 mL diluted sample from each test was spread on potato dextrose agar (PDA) and nutrient agar (NA) media and incubated for 24–48 h at 26 °C for fungi and 37 °C for bacteria. Microbes from mixed cultures were picked and sub-cultured twice on PDA and NA until a pure colony appeared. Pure isolates were kept in PDA and NA slant agar at 4 °C.

### 2.5. Pre-screening of potential fungi decolorizes turquoise blue dye

Turquoise blue textile dye ( $C_{40}H_{25}CuN_9O_{14}S_5$ ) was kindly obtained from the Kombolcha textile industry, where it is currently used. PDA solid media amended with 300-ppm reactive Turquoise blue dye was used to screen fungi having the ability to decolorize due to its clearing zone formation around the mycelia or colony.

### 2.6. Optimization of culture conditions using definitive screening design

A definitive screening design was used in the experiment to select significant operational factors that will provide the optimum dye decolorization and fungal mycelia biomass. Definitive screening designs can work 2–48 factors. The design can be constructed with categorical factors with 2 levels and continuous factors with 3 levels. This research has five parameters, consisting of four continuous factors with three levels, these are temperature (25, 30, 35 °C), pH (4, 7, 10), dye concentration (300, 400, and 500 ppm), inoculum amount (0.5, 0.75, and 1 mL) and one categorical factor with two levels (Two fungi isolates) were set operational factors. Totally fourteen base runs, and 3 levels of each continuous factor and 1 categorical factor with 2 levels were constructed for this investigation. Minitab ver 19 software was used to construct the experimental design. The experimental design layout and measured response was summarized in Table 3.

### 2.7. UV-Vis Spectrophotometer analysis

400 mL of Potato dextrose broth (PDB) was prepared in an Erlenmeyer flask and amended with 300, 400, and 500 ppm Turquoise blue dye separately. In respective of inoculum amounts about  $3 \times 10^8$  spores/mL, fungi was inoculated into each of the experimental run flasks and kept on the shaker for 8 days at 130 rpm. At 48-h intervals, aliquots (5 mL) of culture broth were withdrawn and centrifuged, at 10,000 rpm for 2 min, and the supernatant was used for dye analysis after appropriate dilutions. The PDB medium with turquoise blue at different concentration was used to calibrate for absorbance measurements (Fig. 10.S.4) [32,35]. The Jenway 6405 UV-Vis Spectrophotometer was conducted at 25 °C temperature condition and 620 nm to measure absorbance to determine the dye percentage of degradation at Addis Ababa University Mycology Laboratory. The ability of color decolorization was calculated as the percentage ratio of the decolorized dye concentration to that of the initial one based on the following equation.1 [36]. All experiments were carried out in triplicate.

$$\text{Degradation or Decolorization (\%)} = \frac{C_i - C_f}{C_i} \times 100 \quad \text{eq 1}$$

Where ( $C_i$ ) and ( $C_f$ ) are the initial and final dye concentrations (mg/L) respectively.

### 2.8. Fungi identification using Biolog Microstation

Fungi were grown for 24–48 h at 26 °C on Biolog Universal Agar (BUY). 15 mL of sterile FF inoculum fluid was used to create the inoculum suspension. Pure filamentous fungi were placed into test tubes containing FF inoculum using a sterile cotton swab, and they were adjusted to  $75T \pm 2$  using a biolog turbidimeter. Digital pipettes were used to add 100  $\mu$ L of inoculum to each well of the biolog FF microplate. 96 phenotypic tests make up the FF Micro plate, containing 71 carbon source utilization assays, 23 chemical sensitivity assays, and two controls. The inoculated microplate was incubated at 26 °C for 24–96 h. The FF microplate produces both metabolic product and turbidity growth in order to provide identifications. The FF microplates were read by the Microstation reader at a single 590 nm wavelength. Microstation reader reads the FF micro plate at 24-h to 72-h intervals. The Biolog software, Microlog3 version 4.20.05, compared the results obtained with the test strain to the database and provided identification based on the distance value of the match and the separation score. The recommended and acceptable species identification result must have a similarity index value  $\geq 0.5$  and a probability  $\geq 75\%$  [37]. Fungi species identification by Biolog Microstation was conducted at the Ethiopian Biodiversity Institute, Microbial Biodiversity Directorate Laboratory, and Animal Health Institute, Sebeta; Ethiopia.

## 2.9. Microbial identification using MALDI-TOF

Fungi, and bacteria species identification were carried out using matrix-assisted laser desorption/ionization-time of flight (MALDI-TOF) mass spectrometry (MS) at the Animal Health Institute, Sebeta; Ethiopia. The fungal and bacterial extraction procedure used the method from (Cultivation and sample preparation for filamentous fungi, revision, March 2019, Bruker Daltonics, Bremen, Germany) [38] Bacteria colony and fungi mycelium were transferred into 1 mL double distilled water separately, and pellet by centrifugation. The pellet was again dissolved in 300  $\mu$ L of double distilled water. Then, 900- $\mu$ L ethanol was added and after centrifugation, the ethanol was removed and air-dried. Depending on the size, the pellet was re-suspended in 10–100  $\mu$ L of 70 % formic acid. The same amount of acetonitrile was then added and centrifuged again. Afterwards, 1  $\mu$ L of the supernatant was pipetted onto the MALDI target, overlaid with 1  $\mu$ L of HCCA matrix, and analyzed with MALDI-TOF. The isolates were subjected to MALDI-TOF MS analysis using a Bruker MALDI Biotyper microflex LT (Bruker Daltonics, Bremen, Germany). According to the Bruker recommendations, the level of similarity between an unknown tested specimen and a reference sample is indicated by a log (score), which is referred to as a score  $>1.7$  indicates “highly probable species identification and secure genus identification, a score  $<1.7$  indicates unreliable identification.

## 2.10. Data analysis

The fungus mycelia dry biomass production and percentage of decolorization were analyzed using descriptive and inferential statistics; these were percentage frequency, Mean, and ANOVA analyzed using the Minitab ver 19 statistical software package.

## 3. Results and discussion

### 3.1. Microbial identification at the species level

In this study, a total of 24 fungi and 6 bacterial species were identified using Biolog Microstation and MALDI-TOF (Bruker MALDI Biotyper microflex LT, Bruker Daltonics, Bremen, Germany) from contaminated urban farming soil, effluent, polluted rivers from Addis Ababa and central rift valley area (Table 1 and 2). In similar to this study many researchers have reported different species of microbes identified from polluted sites in different agroecologies. According to Ref. [39] report representatives of the *Actinobacteria*, *Firmicutes*, and *Gamma proteo bacteria* phyla were superior in contaminated soils, whose species are active degraders of xenobiotics in nature [40]. identified 36 microorganisms from heavy metal-contaminated sites in Tangier, Morocco, belonging to the *Aspergillus*, *Penicillium*, *Fusarium*, *Alternaria*, and *Geotrichum* genera that demonstrated high levels of resistance to all metals tested [41]. reported that *Aspergillus fumigates* and *Penicillium chrysogenum* were identified from Azo dye contaminated soil. Ahmad, Ansari and Aqil [42] reported *Aspergillus flavus*, *Aspergillus niger*, *Fusarium oxysporium* and *Penicillium notatum* are isolated fungi from textile contaminated site. Certain bacterial species have been identified from textile waste; these are *Pseudomonas* sp. and *Bacillus* sp [43] Some of the

**Table 1**  
Fungi identified from Addis Ababa urban farming site and central rift valley area in Ethiopia using biolog microstation.

Fungi species	Probability	Similarity	Distance	Specific Area Identified	Sample Type
<i>Y. lipolytica</i>	100 %	0.7	4.46	Addis Ababa, Kera	Cabbage Rhizosphere soil
<i>C. luteolus</i>	100 %	0.64/0.716	4.16/4.27	Addis Ababa, Kera	Cauliflower/Cabbage Rhizosphere soil
<i>R. aurantiaca</i> A	100 %	0.62/0.510/ 0.585	5.80/4.37/ 6.47	Addis Ababa, Saris/Kera& Rift valley	Sald Rhizosphere soil/Farm soil
<i>U. maydis</i>	100 %	0.586	6.31	Addis Ababa, Saris	Sald Rhizosphere soil
<i>T. beigelii</i> B	100 %	0.514	7.68	Ethiopia, Rift valley	Farm soil
<i>C. terreus</i> A	100 %	0.629	5.72	Ethiopia, Rift valley	Forest soil
<i>Z. bailii</i>	98 %	0.65	5.12	Addis Ababa, Kera	Swiss chard Rhizosphere
<i>N. fulvoscens</i>	90 %	0.627	4.61	Addis Ababa, Kera	Swiss chard farm
<i>S. starkeyi-henricii</i>	89 %	0.56	7.83	Addis Ababa, Saris	Cabbage Rhizosphere soil
<i>E. vivi</i>	84 %	0.619	4.03	Addis Ababa, Picoock	Polluted River
<i>R. pustula</i>	–	0.591	4.88	Ethiopia, Rift valley	Lake
<i>C. curvatus</i> A	–	0.401	9.71	Addis Ababa, Saris	Cabbage Rhizosphere soil
<i>R. acheniorum</i>	–	0.265	13.15	Addis Ababa, Kera	Sald Rhizosphere soil
<i>R. sphaerocarum</i>	–	0.337	7.73	Addis Ababa, Kolfe	Carrot Rhizosphere soil
<i>T. atroviride</i> Karsten BGB	97 %	0.83	36.9	Addis Ababa, Kolfe	Sald Rhizosphere soil
<i>E. meridianum</i>	98 %	0.578	35.81	Addis Ababa, Beher Tsege	River water
<i>E. crustaceum</i> Ludwig	99 %	0.58	22.86	Ethiopia, Mojo	Effluent waste
<i>P. roqueforti</i> Thom BCG	99 %	0.701	31.71	Addis Ababa, Lafto	Cabbage Rhizosphere soil
<i>P. lanosum</i> westing	97 %	0.708	20.912	Ethiopia, Mojo	Effluent Waste
<i>A. niger</i> v. Tieggham BGA	98 %	0.701	18.615	Ethiopia, Zewaye	Effluent (Haile resort)
<i>P. heriquei</i>	96 %	0.622	19.66	Addis Ababa, Mekanisa	Cabbage Rhizosphere soil
<i>P. citrinum</i> ThomBCA	98 %	0.507	21.521	Addis Ababa, Goffa Germen	Cabbage Rhizosphere soil
<i>P. janczewkinzi</i> zaleski BGB	100 %	0.588	29.21	Addis Ababa, Kolfe	Cabbage Rhizosphere soil
<i>P. Thom Maire</i> BCG	98 %	0.707	21.54	Addis Ababa, Mekanisa	Salad Rhizosphere soil

**Table 2**  
Bacteria and fungi identified from Addis Ababa urban farming site and central rift valley area using MALDI-TOF

Microbial species	Score Value	Rank	Specific Area	Sample Type
<i>Enterobacter asburiae</i>	2.17	+++	Kality	Sald Rhizospher Soil
<i>Serratia marcescens</i>	2.32	+++	Rift valley	Farm Soil
<i>Serratia nematodiphila</i>	2.43	+++	Rift valley	Farm Soil
<i>Lactobacillus paracasei</i>	2.41	+++	Mekanisa	Brewery Effluent
<i>Klebsiella Aerogenes</i>	2.41	+++	Kera	Swiss chard Rhizospher soil
<i>Geotrichum candidum</i>	2.02	+++	Kolfe	Swiss chard Rhizospher soil
<i>Trichoderma longibranchiatum</i>	2.24	+++	Goffa German	Cabbage Rhizospher soil
<i>Trichoderma orientale</i>	1.9	+	Goffa German	Cabbage Rhizospher soil
<i>Trichoderma koningii</i>	2.18	+++	Saris	Salad Rhizospher soil
<i>Aspergillus niger</i>	1.87	++	Kality	Cabbage Rhizospher soil

Score value > 1.7 indicates “highly probable species identification and secure genus identification, a score <1.7 indicates unreliable identification.

**Table 3**  
DSD experimental design layout and observed response.

StdOrder	RunOrder	PtType	Blocks	Temperature/ <sup>o</sup> C	pH	Dye Conc/ ppm	Inoculums amt/mL	Fungal Isolates	DryBiomass/ g	Decolorization (%)
1	1	2	1	30	10	500	1	<i>P. heriquei</i>	0.668	0
4	2	2	1	25	7	300	1	<i>P. heriquei</i>	0.579	5
6	3	2	1	25	4	400	0.5	<i>P. heriquei</i>	1.594	65
8	4	2	1	25	10	300	0.75	<i>P. citrinum</i>	0.53	70
5	5	2	1	35	10	400	1	<i>P. citrinum</i>	1.821	73
11	6	1	1	35	10	300	0.5	<i>P. heriquei</i>	0.676	1
3	7	2	1	35	7	500	0.5	<i>P. citrinum</i>	1.694	82
9	8	1	1	35	4	300	1	<i>P. citrinum</i>	1.714	8
10	9	1	1	25	10	500	0.5	<i>P. heriquei</i>	0.559	0
14	10	0	1	30	7	400	0.75	<i>P. heriquei</i>	1.792	87
13	11	0	1	30	7	400	0.75	<i>P. citrinum</i>	0.538	15
2	12	2	1	30	4	300	0.5	<i>P. citrinum</i>	1.716	90
7	13	2	1	35	4	500	0.75	<i>P. heriquei</i>	1.626	8
12	14	1	1	25	4	500	1	<i>P. citrinum</i>	1.802	57

commonly identified PAH-degrading bacteria are *P. aeruginosa*, *P. fluorescens*, *Mycobacterium* spp., *Haemophilus* spp., *Rhodococcus* spp., and *Paenibacillus* spp [44]. Fungi of the phylum Ascomycota and Mucoromycota such as *Aspergillus*, *Penicillium*, *Fusarium*, *Trichoderma*, *Acremonium*, and *Cladosporium*, *Mucor*, have been isolated from PAH-contaminated soils, and have the ability to degrade PAHs [45,46].

### 3.2. Pre-screening of potential fungi decolorizes turquoise blue dye

Potato dextrose agar solid media amended with 300 ppm reactive dye is used to screen fungi that have the ability to decolorize due to its clearing zone formation around the colony. Two filamentous fungi were positive in Turquoise blue dye decolorization among twenty four fungi, and selected for further study these were *Penicillium citrinum* Thom BCA and *Penicillium heriquei* (Fig. 8. S.2).

### 3.3. Selection of variables using definitive screening design (DSD) for turquoise blue decolorization

DSD is an experimental design tool used to select the optimum variables that deliver a desired response using a minimum number of

**Table 4**  
Screening Design Model: Percentage Decolorization % Versus Temperature<sup>o</sup>C, pH, Inoculums amt, Dye concentration & Fungal Isolates.

Analysis of Variance.					
Source	DF	Adj SS	Adj MS	F-Value	P-Value
Model	6	14826.1	2471.0	5.46	0.021
Linear	6	14826.1	2471.0	5.46	0.021
Temperature/ <sup>o</sup> C	1	3702.7	3702.7	8.18	0.024
pH	1	2814.8	2814.8	6.22	0.041
Dye conc/ppm	1	589.3	589.3	1.30	0.291
Inoculum amt/mL	1	1988.6	1988.6	4.39	0.074
Fungal isolates	1	1739.3	1739.3	3.84	0.091
Biomass/g	1	8786.7	8786.7	19.41	0.003
**Error	7	3168.9	452.7		
Total	13	17994.9			

runs. The DSD run layout and output are summarized in (Table 3). Among the variables screened, the most influencing factors for decolorization have a high significance level indicated by the Pareto chart and the analysis of variance indicates the significance value. These were temperature (P, 0.024), pH (P, 0.041) and mycelia biomass (P, 0.003) where the p value is  $\leq 0.05$  identified and selected as significant factors for dye removal (Table 4 Fig. 2.S.1). *P. citrinum* and *P. heriquei* were able to decolorizing turquoise blue up to 90 % and 87 % respectively. Different study indicate that the metabolic path of degradation of turquoise blue of the phthalocyanine ring producing sulfophthalimide as the main degradation product, ammonium and Cu(II) ions (Fig. 12.S.6) [47]. In similar to this research different scholars reported [48] *Bacillus megaterium* had good ability to degrade the turquoise blue dye up to 95 % in 10mM/10 mL medium concentration within 48 h [34]. reported that the maximum color removal efficacy of the turquoise blue textile dye with a concentration of 30 mg/L in an aqueous solution was achieved at 78.50 and 85.84 % at pH 6 for *Microporus xanthopus* and *Ganoderma applanatum*, while the color removal efficiency was 82.17 % at pH 5 for *Trametes hirsuta* with seven days of incubation time.

### 3.4. Effect of temperature

Three-temperature conditions, 25, 30, and 35 °C were evaluated for two potential fungal isolates for their ability to decolorize turquoise blue dye. The highest decolorization was 90 % at 30 °C for 300 ppm by *Penicillium citrinum* Thom BCA and 87 % at 30 °C, for 400 ppm by *Penicillium herquei* (Fig. 2) whereas the average mean decolorization of dye by both fungi recorded 48 % at 30 °C (Fig. 6). There is no significant difference in mean biomass production and mean percentage decolorization within the temperature ranges (Table 5). Different studies have been done for Azodye decolorization in different temperature ranges [49]. reported that the decolorizing rate of Turquoise Blue HFG by *Lysinibacillus fusiformis* B26 increased from about 36.02 % to 53.63 % when the temperature increased from 40 °C to 50 °C, respectively. The optimum decolorizing activity for this dye was at 50 °C. Another report by [48], the biodegradation of Turquoise Blue by *B. megaterium* the optimum temperature was 37 °C. Turquoise Blue dye maximum decolorization of was achieved by *Trichoderma harzianum* 94 % at the temperature of 37 °C, a dye concentration of 50 mg/L and 0.4 g/L biosorbent dose [50]. [51] conducted research on the decolorization of acid blue 29, disperse red 1, and congo red by several indigenous *Aspergillus* fungal strains and optimal conditions dye decolorization was 86 % by *A. flavus*, *A. fumigatus*, *A. niger*, and *A. terreus* was recorded at 30–35 °C at pH 7 [32]. studied the biodecolorization of an Azo dye, eriochrome black T by *Penicillium citrinum*. The results showed that optimum decolorization was 98 % at a concentration of 10 mg/L, 35 °C and pH 6 for 5 days. However, there is an Azo dye concentration difference in this study *Penicillium citrinum* able to decolorize up 90 % at 30 °C for 300 ppm of Turquoise Blue dye. Another study by Ref. [52] on the third day of incubation, *A. nomius* and *A. aculeatus* demonstrated 98 % decolorization of CI Direct Blue 201 textile dye (50 mg/L) with the addition of modified Kirk's medium. In this study, both fungal strains were proven to be more effective for dye decolorization at temperatures ranging from 25 °C to 35 °C.

### 3.5. Effect of pH

The effect of pH was evaluated at pH 4, 7 and 10 for percentage decolorization. The result revealed that *Penicillium citrinum* Thom BCA recorded the highest decolorization of turquoise blue is 90 % at pH 4 and 87 % decolorization by *Penicillium herquei* at pH 7 (Table .3, Fig. 3) and Whereas average mean percentage decolorization was 47.2 % recorded at pH 7 by both fungi (Table, 6). Similar to this study [48], reported that the maximum decolorization of turquoise blue dye by *Bacillus megaterium* species within 48 h at pH 7. According to Ref. [49] the *L. fusiformis* B26 strain could decolorize turquoise blue within a broad pH range 6.0–10.0; the optimum pH was 7.5. The Turquoise Blue dye maximum decolorization of was achieved by *Trichoderma harzianum* 94 % at pH 4, a dye

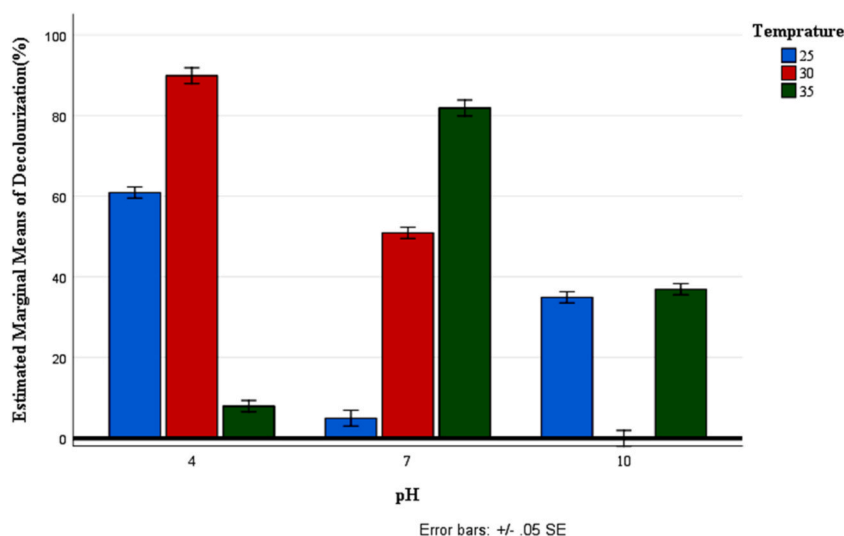


Fig. 2. The effect of Temperature and pH on percentage of Turquoise blue dye decolorization.



**Table 5**

The effect of Temperature (°C) for mean fungal biomass and Turquoise blue dye decolorization(%).

Temperature(°C)	Fungal Biomass (Mean ± Std. Error)	Dye Decolorization(%) (Mean ± Std. Error)
25	1.013 ± 0.28 <sup>a</sup>	39.40 ± 15.2 <sup>a</sup>
30	1.179 ± 0.33 <sup>a</sup>	48.00 ± 23.6 <sup>a</sup>
35	1.506 ± 0.21 <sup>a</sup>	34.40 ± 17.7 <sup>a</sup>

\*Superscript of the same letter not significant across column.

**Table 6**

The effect of pH on mean fungal biomass and Turquoise blue dye decolorization(%).

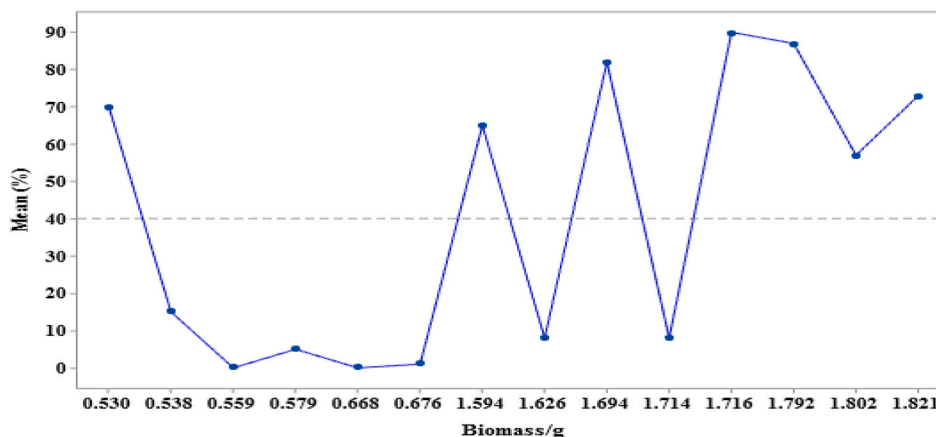
pH	(Biomass/g) Mean ± Std. Error	(Decolorization (%)) Mean ± Std. Error
4	1.690 ± 0.219 <sup>a</sup>	45.600 ± 17.581 <sup>a</sup>
7	1.151 ± 0.244 <sup>a</sup>	47.250 ± 19.657 <sup>a</sup>
10	0.851 ± 0.219 <sup>a</sup>	28.800 ± 17.581 <sup>a</sup>

\*Superscript of the same letter not significant across column.

concentration of 50 mg/L, and 0.4 g/L biosorbent dose [50]. According to Ref. [34] the optimal color removal efficiency of the turquoise blue textile dye with a concentration of 30 mg/L in an aqueous solution was achieved at 78.50 and 85.84 % at pH 6 by *Microporus xanthopus* and *Ganoderma applanatum*, respectively, while *Trametes hirsuta* achieved 82.17 % at pH 5 after seven days of incubation. In this study, pH4 and pH7 were effective for *Penicillium citrinum* Thom BCA and *Penicillium herquei* for the decolorization of turquoise blue up to 90 % & 87 % respectively. In one study, by Ref. [48] *Bacillus megaterium* species gave maximum decolorization of turquoise blue dye within 48 h at pH 7 and 37 °C [53]. Study on decolorization and biodegradation of reactive blue by *Aspergillus* sp., the result indicates that the highest color removal was detected in acidic pH 3 (98 %) when compared to neutral and basic pH [54]. Also carried out the study on degradation of crystal violet dye by *Aspergillus niger*. The decolorization process was effective (up to 84.6 %) with the examined range of dye concentrations (10–40) ppm at pH 5.5 and dry mycelia biomass (0.47 g/100 mL). However, the pH requirement depends on microbial species, in this study, for both fungi species pH 4 and 7 was recorded as the effective decolorization point.

### 3.6. The effect of mycelia biomass

Fungal mycelia biomass grown in the potato dextrose broth (PDB) was harvested, and dry weight was measured. The highest decolorization of Turquoise blue was recorded 90 % at 1.716 g fungal dry mycelia biomass production (Figs. 3 and 4). In this study, the fungi were compared with other filamentous fungal biomass production study and the ability of azodye decolorization, its biomass production is very good. According to Ref. [55] when complete decolorization of turquoise blue had occurred. Fungal biomass production was found to increase up to a maximum of 2.2 g/L at day 6 after incubation. According to Ref. [56] study on *Aspergillus niger* showed that 95.84 % efficient decolorization of yellow (PY-74) and reactive blue (RB-38) with 0.2–0.26 g/100 mL fungal dry biomass production with 3–4 days of incubation, and *Fusarium* sp. and *Rhizopus* sp. were the second active fungi in decolorization, with growth rates ranging from 0.11 to 0.16 g/100 mL. However, fungal biomass production depends on different factors, the presence of chitin, and melanin influences the ability of fungi to act as sorbents or sequesters [57]. Fungal biomass is very useful for sequestering or producing

**Fig. 3.** Main effects plot of fungal mycelia biomass for percentage decolorization

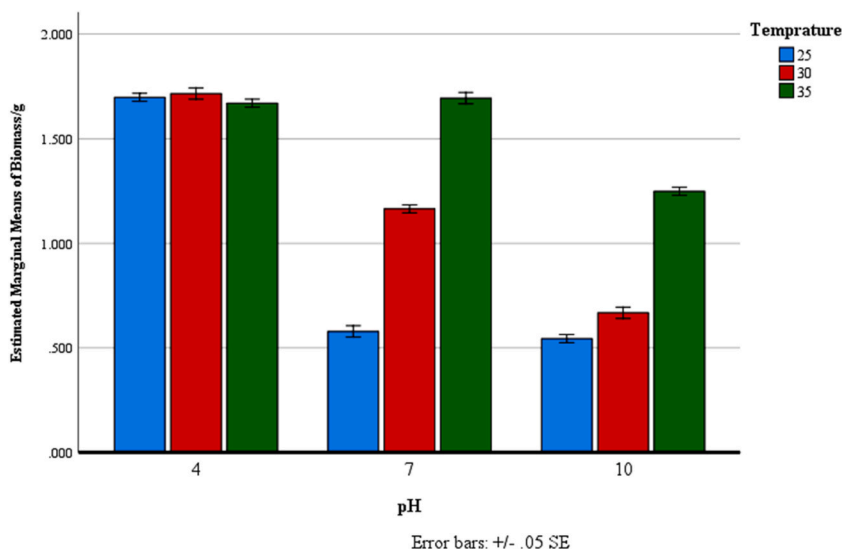


Fig. 4. The effect of pH and Temperature in fungal biomass production

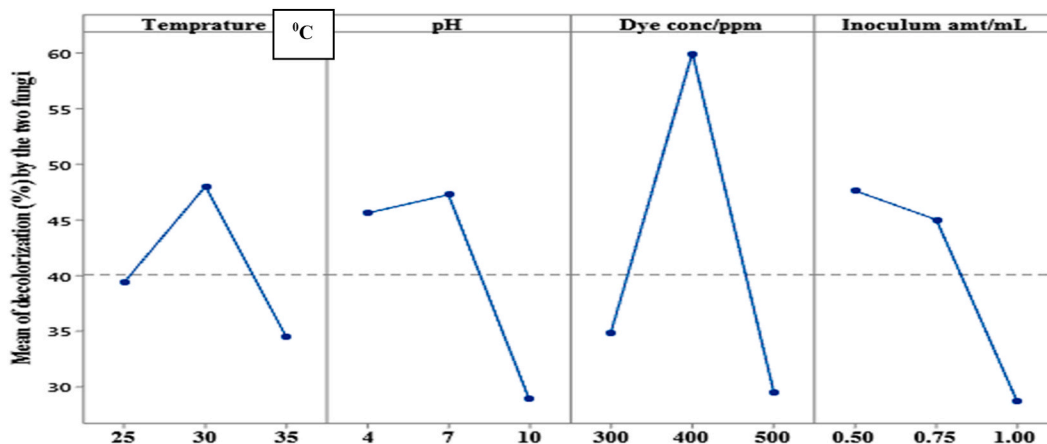


Fig. 5. Main effects plot for average mean percentage decolorization by the two fungi isolates (*Penicillium citrinum* Thom BCA and *Penicillium herquei*)

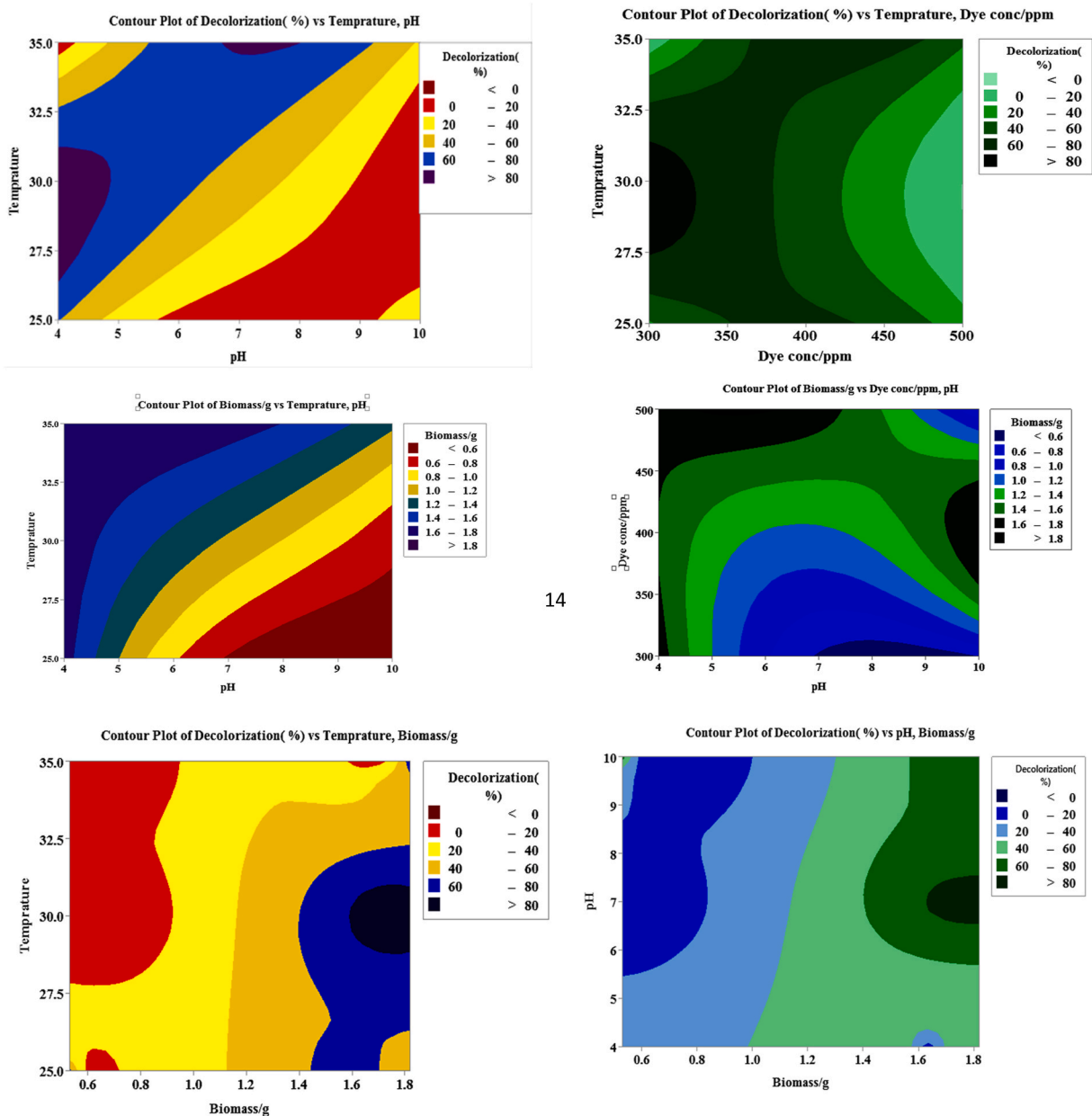
of enzymes involved in biodegradation/biotransformation [58].

#### 4. Main and interaction effect of independent variables on turquoise blue dye decolorization

##### 4.1. Graphical analysis of the model

A main effect is the effect of one independent variable on the dependent variable. Each variable (pH,  $T^{\circ}$ , dye concentration and fungi isolates) were evaluated separately for their effect on the percentage of decolorization and biomass production of both fungi isolates, the result showed that average mean values of the two fungi together for each variables are summarized in Fig. 5. The interaction effects include simultaneous effects of two or more variables on the process response. Interaction occurs when the effect of one independent variable changes depending on the level of another independent variable. Interaction is a complex multivariable effect which provides more precise information than a simplified main effect. Contour plots are used to display the relationship and interaction between independent variables and a dependent variable. The contour plots, presented by two and three dimensional graphs using color removal based on the simultaneous variation of strain composition from 0 to 100 % for each strain. The counter plot also described the individual and cumulative effect of these two variables with *Penicillium herquei* and *Penicillium citrinum* ThomBCA) their subsequent effects on the response of color removal (Fig.9.S.3) The result indicated in this study is that the interaction of pH and temperature, above 80 % decolorization, occurred 25–30 °C and pH 4–7. The interaction of pH and fungal mycelia biomass, above 80 % of color removal, was indicated at pH 7 and 1.7–1.8 Biomass/g. The interaction effect of temperature and pH for mycelia biomass production was above 1.8 biomass/g is recorded at pH 4–7 and 25–35 °C (Fig. 6).





14

**Fig. 6.** Interaction Effects of Independent Variables by contour plots graphical analysis (Temperature (°C)\*pH, Temperature (°C)\* Dye concentration, pH\* Dye concentration for Dye decolorization (%)

#### 4.2. Response optimization

Response optimization helps to identify the combination of variables effect that jointly optimize a single response or a set of responses. This is useful when researchers need to evaluate the impact of multiple variables on a response. In this study, the optimal values were obtained from the combined effect of three parameters (Temperature, pH and concentration of the dye).The optimum dye decolorization result revealed that Temperature is 25 °C, pH, 4, inoculums amount, 0.5, and dye concentration 300 ppm(Fig. 11. S.5).

#### 5. Conclusion

Environmental pollution due to the release of untreated Azo dye waste effluent from different industries is causing numerous health and environmental damages. Due to some limitations of physicochemical treatment method alone did not solve the problem and contemporary civilizations continue to strive in search for alternative treatment strategies for Azo dye contamination. This study was

aimed at screening and identifying potential microbes from polluted environments for bioremediation purposes. In this study, 24 fungi and 6 bacterial species were identified at species level from polluted sites of urban farming area, rivers, industry effluent, central rift valley farm soil, and lakes in Ethiopia. Turquoise blue dye is one of largely used reactive dye for most textile industries and selected for microbial degradation studies. In this work, two potential filamentous fungi were selected for Turquoise blue dye removal ability using UV-Vis Spectrophotometer absorbance measurement only, these are *Penicillium citrinum* Thom BCA recorded 90 % dye removal at pH 4 and 30 °C and *Penicillium herquei*, have shown 87 %, Turquoise reactive blue dye decolorization ability at pH 7, and 30 °C. The highest fungal mycelial biomass harvested, 1.716 g at 90 % dye removal by *Penicillium citrinum* Thom BCA. This helps to determine the fungi biomass large production for inoculants production using different locally available substrate. The study limitation was microbial dye decolorization research focusing only one type of Azo dye and microbial consortia study was not carried out due to availability high technology facilities. Therefore, these two fungi are good candidates for Turquoise blue dye removal ability based on UV-Vis spectrophotometer absorbance measurements, in mycoremediation applications. I recommend further dye degradant analysis by using HPLC, FTIR, GCMS are required according to the availability of the technology, as well as toxicity analysis at greenhouse and field evaluation are needed for bioinoculant formulation on a large scale. Multiple Azodye degradation studies using concertina of microbes are also required for full scale microbial remediation treatment strategies implementation.

## Funding

This work was supported by Addis Ababa University student research fund and Ethiopian Biodiversity Institute Microbial Biodiversity Directorate in kind support.

## Ethical approval

Not applicable.

## Data availability

All data generated or analyzed during this study are included in article/supp. material/referenced in this article with the repository and the accession number HELIYON-D-23-56772.

## CRedit authorship contribution statement

**Birhanu Gizaw:** Methodology, Investigation, Formal analysis, Data curation, Conceptualization, Writing – original draft. **Tesfaye Alemu:** Writing – review & editing, Supervision, Investigation. **Girma Ebsa:** Methodology, Investigation.

## Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Birhanu Gizaw reports equipment, drugs, or supplies was provided by Addisababa University and Ethiopian Biodiversity Institute Microbial Biodiversity Directorate. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgment

The author acknowledged the Animal Health Institute, Sebeta Ethiopia, especially Dr. Abebe Olani & Dr. Shumbisa facilitate microbial identification. I also appreciate Kombolcha Textile Industry kindly offering Azodye for this experiment. Addis Ababa urban area farmers willing to give soil samples from their farm, Addis Ababa University Mycology Laboratory facility and Biodiversity Institute Microbial Directorate, and research team, for the technical and unreserved support.

## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.heliyon.2024.e32769>.

## References

- [1] C.C. Okafor, C.N. Madu, C.C. Ajaero, J.C. Ibekwe, C.A. Nzekwe, C. Okafor, C. Madu, C. Ajaero, J. Ibekwe, C. Nzekwe, Sustainable management of textile and clothing, *Clean Technol. Recycl* 1 (2021) 70–87, <https://doi.org/10.3934/ctr.2021004>.
- [2] A.S. Ademe, M. Alemayehu, Source and determinants of water pollution in Ethiopia: distributed lag modeling approach, *Intellectual Property Rights* 2 (2014) 110, <https://doi.org/10.4172/2375-4516.1000110>.
- [3] P. Restiani, *Water governance mapping report: textile industry water use in Bangladesh*, Sweden Textile Water Initiative (2016) 1–49.

- [4] A.K. Mehari, S. Gebremedhin, B. Ayele, Effects of Bahir Dar textile factory effluents on the water quality of the head waters of Blue Nile River, Ethiopia, *International journal of analytical chemistry* (2015) 2015, <https://doi.org/10.1155/2015/905247>.
- [5] D. Dadi, T. Stellmacher, F. Senbeta, S. Van Passel, H. Azadi, Environmental and health impacts of effluents from textile industries in Ethiopia: the case of Gelan and Dukem, Oromia Regional State, Environ. Monit. Assess. 189 (2017) 1–30, <https://doi.org/10.1007/s10661-016-5694-4>.
- [6] A.A. Damtew, A.A. Ketema, B.M. Behailu, Assessment of industrial effluent pollution on Borkena River, Kombolcha, Ethiopia, *advances of science and technology: 7th EAI international conference, ICAST 2019, Bahir dar, Ethiopia, august 2–4, 2019, Proceedings 7*, Springer (2020) 325–333, [https://doi.org/10.1007/978-3-030-43690-2\\_22](https://doi.org/10.1007/978-3-030-43690-2_22).
- [7] A. Gürses, M. Açıkıldız, K. Güneş, M.S. Gürses, A. Gürses, M. Açıkıldız, K. Güneş, M.S. Gürses, Dyes and pigments: their structure and properties, *Dyes Pigments* (2016) 13–29, [https://doi.org/10.1007/978-3-319-33892-7\\_2](https://doi.org/10.1007/978-3-319-33892-7_2).
- [8] O. Lipskikh, E. Korotkova, Y.P. Khristunova, J. Barek, B. Kratochvil, Sensors for voltammetric determination of food azo dyes-A critical review, *Electrochim. Acta* 260 (2018) 974–985, <https://doi.org/10.1016/j.electacta.2017.12.027>.
- [9] M. Shah, Effective treatment systems for azo dye degradation: a joint venture between physico-chemical & microbiological process, *International Journal of Environmental Bioremediation & Biodegradation* 2 (2014) 231–242, <https://doi.org/10.12691/ijebb-2-5-4>.
- [10] K. Maheshwari, M. Agrawal, A. Gupta, Dye pollution in water and wastewater, Novel materials for dye-containing wastewater treatment, 1–25, <https://doi.org/10.1007/978>, 2021.
- [11] P. Lissy, M. Sreeja, Utilization of sludge in manufacturing energy efficient bricks, *IOSR Journal of Mechanical and Civil Engineering (IOSR-JMCE)* (2014) e-ISSN. 2278-1684.11(4).70-73.
- [12] O.S. Oladejo, S.O. Dahunsi, A.T. Adesulu-Dahunsi, S.O. Ojo, A.I. Lawal, E.O. Idowu, A.A. Olanipekun, R.A. Ibikunle, C.O. Osueke, O.E. Ajayi, Energy generation from anaerobic co-digestion of food waste, cow dung and piggery dung, *Bioresour. Technol.* 313 (2020) 123694, <https://doi.org/10.1016/j.biortech.2020.123694>.
- [13] G. Delelegn, Assessment of physical and chemical contents of textile sludge and associated risks on public health: in Case of Common Effluent Treatment Plant (CETP), *Res Rev J Ecol Environ Sci* 6 (2018) 21–26.
- [14] S. Keerthana, K. Kavya, T. Pradeep, S. Sharmila, Study on effect of partial replacement of sludge in bricks, *Int. J. Recent Technol. Eng.* 7 (2019) 364–369.
- [15] V. Kumar, R.D. Parihar, A. Sharma, P. Bakshi, G.P.S. Sidhu, A.S. Bali, I. Karaouzas, R. Bhardwaj, A.K. Thukral, Y. Gyasi-Agyei, Global evaluation of heavy metal content in surface water bodies: a meta-analysis using heavy metal pollution indices and multivariate statistical analyses, *Chemosphere* 236 (2019) 124364, <https://doi.org/10.1016/j.chemosphere.2019.124364>.
- [16] V. Palanisamy, Utilization of textile effluent waste sludge in brick production, *Int. J. Sci. Basic Appl. Res.* 4 (2011) 1–10.
- [17] D.A. Beshah, G.A. Tiruye, Y.S. Mekonnen, Characterization and recycling of textile sludge for energy-efficient brick production in Ethiopia, *Environ. Sci. Pollut. Control Ser.* 28 (2021) 16272–16281, <https://doi.org/10.1007/s11356-020-11878-7>.
- [18] I.M. Banat, P. Nigam, D. Singh, R. Marchant, Microbial decolorization of textile-dyecontaining effluents: a review, *Bioresour. Technol.* 58 (1996) 217–227, [https://doi.org/10.1016/S0960-8524\(96\)00113-7](https://doi.org/10.1016/S0960-8524(96)00113-7).
- [19] H. Lu, J. Zhou, J. Wang, W. Si, H. Teng, G. Liu, Enhanced biodecolorization of azo dyes by anthraquinone-2-sulfonate immobilized covalently in polyurethane foam, *Bioresour. Technol.* 101 (2010) 7185–7188, <https://doi.org/10.1016/j.biortech.2010.04.007>.
- [20] H. Modi, G. Rajput, C. Ambasana, Decolorization of water soluble azo dyes by bacterial cultures, isolated from dye house effluent, *Bioresour. Technol.* 101 (2010) 6580–6583, <https://doi.org/10.1016/j.biortech.2010.03.067>.
- [21] J.-S. Bae, H.S. Freeman, S.D. Kim, Influences of new azo dyes to the aquatic ecosystem, *Fibers Polym.* 7 (2006) 30–35, <https://doi.org/10.1007/BF02933599>.
- [22] B.T. Wagaye, G.A. Walle, Overview of Ethiopian textile industry, *Journal of textiles and polymers* 6 (2018) 117–120.
- [23] P. Pal, *Industrial Water Treatment Process Technology*, Butterworth-Heinemann, 2017.
- [24] I. Mnif, S. Maktouf, R. Fendri, M. Kriaa, S. Ellouze, D. Ghribi, Improvement of methyl orange dye biotreatment by a novel isolated strain, *Aeromonas veronii* GRI, by SPB1 biosurfactant addition, *Environ. Sci. Pollut. Control Ser.* 23 (2016) 1742–1754, <https://doi.org/10.1007/s11356-015-5294-9>.
- [25] T. Vimala, T. Poonghuzhalij, Estimation of pigments from seaweeds by using acetone and DMSO, *Int. J. Sci. Res.* 4 (2015) 1850–1854.
- [26] J. Derco, A.Ž. Gotvajn, O. Čižmarová, J. Dudáš, L. Sumegová, K. Šimovičová, Removal of micropollutants by ozone-based processes, *Processes* 9 (2021) 1013, <https://doi.org/10.3390/pr9061013>.
- [27] J. Korenak, J. Ploder, J. Trček, C. Hélix-Nielsen, I. Petrinic, Decolorisations and biodegradations of model azo dye solutions using a sequence batch reactor, followed by ultrafiltration, *Int. J. Environ. Sci. Technol.* 15 (2018) 483–492, <https://doi.org/10.1007/s13762-017-1406-z>.
- [28] W.M. Abd El-Rahim, H. Moawad, A.Z.A. Azeiz, M.J. Sadowsky, Optimization of conditions for decolorization of azo-based textile dyes by multiple fungal species, *J. Biotechnol.* 260 (2017) 11–17, <https://doi.org/10.1016/j.jbiotec.2017.08.022>.
- [29] W.M. Abd El-Rahim, A.Z.A. Azeiz, H. Moawad, M.J. Sadowsky, Identification and characterization of two peroxidases from *Lichtheimia corymbifera*, *Biocatal. Agric. Biotechnol.* 18 (2019) 100995, <https://doi.org/10.1016/j.bcab.2019.01.033>.
- [30] P. Puranik, K. Paknikar, Biosorption of lead and zinc from solutions using *Streptovorticillium cinnamoneum* waste biomass, *J. Biotechnol.* 55 (1997) 113–124, [https://doi.org/10.1016/S0168-1656\(97\)00067-9](https://doi.org/10.1016/S0168-1656(97)00067-9).
- [31] P.A. Ramalho, M.H. Cardoso, A. Cavaco-Paulo, M.T. Ramalho, Characterization of azo reduction activity in a novel ascomycete yeast strain, *Appl. Environ. Microbiol.* 70 (2004) 2279–2288, <https://doi.org/10.1128/AEM.70.4.2279-2288.2004>.
- [32] P.O. Bankole, A.A. Adekunle, O.F. Obidi, V.V. Chandanshive, S.P. Govindwar, Biodegradation and detoxification of Scarlet RR dye by a newly isolated filamentous fungus, *Peyronella prospodis*, *Sustainable Environment Research* 28 (2018) 214–222, <https://doi.org/10.1016/j.serj.2018.03.001>.
- [33] D. Gashaye, Wastewater-irrigated urban vegetable farming in Ethiopia: a review on their potential contamination and health effects, *Cogent Food Agric.* 6 (2020) 1772629, <https://doi.org/10.1080/23311932.2020.1772629>.
- [34] I.K. Sudiana, D.M. Citrawathi, I. Sastrawidana, S. Maryam, I.N. Sukarta, G.A.H. Wirawan, Biodegradation of turquoise blue textile dye by wood degrading local fungi isolated from a plantation area, *Journal of Ecological Engineering* 23 (2022), <https://doi.org/10.12911/22998993/150044>.
- [35] S.-O. Yang, H. Sodaneath, J.-I. Lee, H. Jung, J.-H. Choi, H.W. Ryu, K.-S. Cho, Decolorization of acid, disperse and reactive dyes by *Trametes versicolor* CBR43, *Journal of Environmental Science and Health, Part A* 52 (2017) 862–872, <https://doi.org/10.1080/10934529.2017.1316164>.
- [36] G. Guo, X. Li, F. Tian, T. Liu, F. Yang, K. Ding, C. Liu, J. Chen, C. Wang, Azo dye decolorization by a halotolerant consortium under microaerophilic conditions, *Chemosphere* 244 (2020) 125510, <https://doi.org/10.1016/j.chemosphere.2019.125510>.
- [37] M. McGinnis, T. Molina, D. Pierson, S. Mishra, Evaluation of the Biolog MicroStation system for yeast identification, *J. Med. Vet. Mycol.* 34 (1996) 349–352, <https://doi.org/10.1080/02681219680000591>.
- [38] C. Honsig, B. Selitsch, M. Hollenstein, M.G. Vossen, K. Spettel, B. Willinger, Identification of filamentous fungi by MALDI-TOF mass spectrometry: evaluation of three different sample preparation methods and validation of an in-house species cutoff, *Journal of Fungi* 8 (2022) 383, <https://doi.org/10.3390/jof8040383>.
- [39] T. Doolotkeldieva, M. Konurbayeva, S. Bobusheva, Microbial communities in pesticide-contaminated soils in Kyrgyzstan and bioremediation possibilities, *Environ. Sci. Pollut. Control Ser.* 25 (2018) 31848–31862, <https://doi.org/10.1007/s11356-017-0048-5>.
- [40] L. Ezzouhri, E. Castro, M. Moya, F. Espinola, K. Lairini, Heavy metal tolerance of filamentous fungi isolated from polluted sites in Tangier, Morocco, *Afr. J. Microbiol. Res.* 3 (2009) 35–48.
- [41] A. Hassana, B.T. Vincent, I.M. Nasiru, N. Yakubu, M.F. Gogo, U.H. Boko, Molecular identification of azo dye degrading fungi isolated from azo dye contaminated soil of local dyeing facility in Bida, Niger state, *Indian Journal of Pure & Applied Biosciences* 7 (2019) 1–7, <https://doi.org/10.18782/2320-7051.7641>.
- [42] I. Ahmad, M.I. Ansari, F. Aqil, Biosorption of Ni, Cr and Cd by metal tolerant *Aspergillus niger* and *Penicillium* sp, using single and multi-metal solution 44 (1) (2006) 73–76.
- [43] S.A.J. Rima, G.K. Paul, S. Islam, M. Akhtar-E-Ekram, S. Zaman, M.A. Saleh, M.S. Uddin, Efficacy of *Pseudomonas* sp. and *Bacillus* sp. in textile dye degradation: a combined study on molecular identification, growth optimization, and comparative degradation, *Journal of Hazardous Materials Letters* 3 (2022) 100068, <https://doi.org/10.1016/j.hazl.2022.100068>.

- [44] A. Ding, Y. Sun, J. Dou, L. Cheng, L. Jiang, D. Zhang, X. Zhao, Characterizing Microbial Activity and Diversity of Hydrocarbon-Contaminated Sites, *Hydrocarbon* (2013) 18.
- [45] O. Potin, C. Rafin, E. Veignie, Bioremediation of an aged polycyclic aromatic hydrocarbons (PAHs)-contaminated soil by filamentous fungi isolated from the soil, *Int. Biodeterior. Biodegrad.* 54 (2004) 45–52, <https://doi.org/10.1016/j.ibiod.2004.01.003>.
- [46] P. Godoy, R. Reina, A. Calderón, R.-M. Wittich, I. García-Romera, E. Aranda, Exploring the potential of fungi isolated from PAH-polluted soil as a source of xenobiotics-degrading fungi, *Environ. Sci. Pollut. Control Ser.* 23 (2016) 20985–20996, <https://doi.org/10.1007/s11356-016-7257-1>.
- [47] T. Marchis, P. Avetta, A. Bianco-Prevot, D. Fabbri, G. Viscardi, E. Laurenti, Oxidative degradation of remazol turquoise blue G 133 by soybean peroxidase, *J. Inorg. Biochem.* 105 (2011) 321–327, <https://doi.org/10.1016/j.jinorgbio.2010.11.009>.
- [48] B. Joshi, K. Kabariya, S. Nakrani, A. Khan, F.M. Parabia, H.V. Doshi, M.C. Thakur, Biodegradation of turquoise blue dye by *Bacillus megaterium* isolated from industrial effluent, *Am. J. Environ. Protect.* 1 (2013) 41–46, <https://doi.org/10.12691/env-1-2-5>.
- [49] N.M. Dogan, N. Bozbeyoglu, D. Arar, H.A. Akdogan, M.C. Topuz, Y. Beyatli, Investigation of reactive dye Turquoise blue HFG removal with *Lysinibacillus fusiformis* B26 and detection of metabolites, *Fresenius Environ. Bull.* 22 (2013) 2567–2575.
- [50] V. Karthik, K. Saravanan, E. Nakkeeran, N. Selvaraju, Biosorption of Turquoise Blue dye from aqueous solution by dried fungal biomass (*Trichoderma harzianum*)-kinetic, isotherm and thermodynamic studies, *Desalination Water Treat.* 74 (2017) 362–370.
- [51] F. Ameen, T.M. Dawoud, F. Alshehrei, K. Alsamhary, A. Almansob, Decolorization of acid blue 29, disperse red 1 and Congo red by different indigenous fungal strains, *Chemosphere* 271 (2021) 129532, <https://doi.org/10.1016/j.chemosphere.2021.129532>.
- [52] E. Ekanayake, P. Manage, Decolourisation and Detoxification of CI Direct Blue 201 Textile Dye by Two Fungal Strains of Genus *Aspergillus*, 2020, <https://doi.org/10.4038/jnsfr.v48i1.9935>.
- [53] M. Ramya, B. Anusha, S. Kalavathy, S. Devilaksmi, Biodecolorization and biodegradation of reactive blue by *Aspergillus* sp, *Afr. J. Biotechnol.* 6 (2007) 2917–2926, <https://doi.org/10.1007/s13762-016-1117-x>.
- [54] H. Ali, S. Shehata, K. Ramadan, Microbial decolorization and degradation of crystal violet dye by *Aspergillus niger*, *Int. J. Environ. Sci. Technol.* 13 (2016) 2917–2926, <https://doi.org/10.1007/s13762-016-1117-x>.
- [55] A. Conneely, W. Smyth, G. McMullan, Metabolism of the phthalocyanine textile dye remazol turquoise blue by *Phanerochaete chrysosporium*, *FEMS Microbiol. Lett.* 179 (1999) 333–337, <https://doi.org/10.1111/j.1574-6968.1999.tb08746.x>.
- [56] M. Mazaheri Tehrani, M. Mazaheri Assadi, H. Rashedi, Biodecolorization of textile effluents by autochthonous fungi, *Journal of Applied Biotechnology Reports* 1 (2014) 161–165.
- [57] G. Gadd, Fungi and their role in the biosphere, *Encyclopedia of ecology*, Elsevier (2008) 1709–1717, <https://doi.org/10.1016/B978-008045405-4.00734-5>.
- [58] W. Przystaś, E. Zablocka-Godlewska, E. Grabińska-Sota, Efficiency of decolorization of different dyes using fungal biomass immobilized on different solid supports, *Braz. J. Microbiol.* 49 (2018) 285–295, <https://doi.org/10.1016/j.bjm.2017.06.010>.