Mycoremediation of textile effluent: A toxicological evaluation and its possible correlation with COD

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Abstract

Globally, textile industries are one of the major sectors releasing dye pollutants. This is the first report on the positive correlation between toxicity and COD of textile effluent along with the proposed pathway for enzymatic degradation of acid orange 10 using \textit{Geotrichum candidum} within a very short stretch of time (18h). Removal efficiency of this mycoremedial approach after 18 h in terms of color, dye concentration as well as reduction of chemical oxygen demand (COD) and biological oxygen demand (BOD) in the treated effluent reached to 89\%, 87\%, 98.5\% and 96.3\% respectively. FT-IR analysis of the treated effluent confirmed biodegradation. The LC-MS analysis showed the degradation of acid orange 10, which was confirmed by the formation of two biodegradation products, 7-oxo-8-iminonaphthalene-1,3-disulfonate and nitrosobenzene, which subsequently undergoes stepwise hydrogenation and dehydration to form aniline via phenyl hydroxyl amine as intermediate. The X-ray diffraction (XRD) studies showed that heavy metals content in the treated effluent has reduced along with decrease in % crystallinity, indicating biodegradation. The connection between toxicity and COD was also inveterated using Pearson’s correlation coefficient. Further the toxicological studies indicated the toxicity of raw textile effluent and relatively lower toxic nature of metabolites generated after biodegradation by \textit{G. candidum}.

Graphical Abstract
1. Introduction

Urbanization and industrialization have paved the path for development of the many industries, including the textile industries. Clothing and textiles, after agriculture, is the basic requirement of human being. While the textile industry contributes worldwide economically, the environmental effects are due to high volumes of water use and the diversity and quantities of chemicals that are used in all manufacturing phases of textiles. The untreated effluent when disposed in the water bodies seriously impacted the people in the area (Agrawal and Verma 2019). Rivers and drainage bodies get loaded with precarious textile effluents that impact on the water quality, the aquatic organisms and human life (Kumar et al. 2013, 2014, 2015a, b; Singh and Balomajumder 2016; Mishra et al. 2016). The diverse sort of dyes and chemicals used in textile manufacturing makes textile effluents very complex in terms of chemical compositions. According to previous records, in addition to dyes and its auxiliaries over 8000 chemicals are added such as several acids, salts, surfactants, metals, oxidizing and reducing agents (Reddy and Osborne 2020). These
Recalcitrants in untreated effluents are both harmful to marine and terrestrial organisms and have prolonged effects on health (Hamidi et al., 2014).

To assess the performance of wastewater treatment facilities, the influent and treated water samples after each treatment phase (physical, chemical and biological) should be tracked. In general, microbial degradation is known to be a safe, natural, inexpensive and effective pollutant removal technique in the world (Mishra et al. 2016; Singh et al. 2017, 2018; Kumar et al. 2017). The eco-friendliness and low cost of biological technology has in particular gained significant attention (Maljai et al. 2009). However, bacteria typically contribute to the breakdown of textile dyes creating and accumulating more intractable or hazardous aromatic amine substances that restrict their comprehensive applications to azo dye wastewater treatment plants (Davies et al. 2006). Enhanced techniques are evidently the pre-condition for accelerated elimination of azo dyes, because any residual contaminants should be removed completely. Fungi have been investigated, especially those secreting non-specific oxidases which eventually lead to the azo dyes mineralization into CO₂ (Wanderley et al. 2018). Fungal degradation (Mycoremediation) also leads to complete decoloration and detoxification, which prevents sludge removal and secondary contamination issues.

Majority of studies based on toxicity assessments could be done with bioassays using all forms of harmful compounds found in textile effluent and may well be utilized to evaluate the effect of unidentified compounds which cause detrimental, additive and synergistic effects (Ma et al. 2016; Yu et al. 2014).

Geotrichum sp. out of the few fungi have been observed for degradation of large quantity of artificial colors and molasses (Kim and Shoda 1998, 1999; Chen and Zhao 2008; Shintani and Shoda 2013). Since, Geotrichum sp have not been explored much, therefore it is being used in the current study for the biodegradation and detoxification of textile effluent. The analysis of conventional parameters of textile effluent (before and after mycoremediation) and the inter-relationship between toxicity and COD have been carried out in this study. In an attempt to validate the non-toxicity of treated effluent, the biodegradation analysis such as FT-IR, LC-MS and XRD have been conducted. Furthermore, bioassays such as genotoxicity, phytotoxicity and microbial toxicity assays have also been carried out so that the toxicity level of raw and treated textile effluent can be assessed.

2. Materials and Methods

2.1. Collection of samples

Samples of textile dyeing effluent have been obtained from a nearby textile factory in Khurda, India. During regular operations, factory workers sampled 100mL of wastewater every two hours to ensure that the study involves variability in substances.

2.2. Microbial culture conditions

This work utilizes Geotrichum candidum, a ubiquitous fungus belonging to Dipodoascaceae family. It was grown at 35 °C on Potato Dextrose Agar plates (PDA) (pH-5.6 ± 0.2). Pure fungal culture was inoculated in 3 percent malt extract broth after 24 hours to maintain the strain and cultured at 35 °C, 100 rpm. Using DP media (dextrose and peptone in ratio of 2:3) at 35 °C and 100 rpm, the optimum fungal growth was achieved (Rajhans et al. 2019).

2.3. Analysis of conventional indicators of textile effluent

In this part of the analysis, the G. candidum culture was used to biodegrade the textile effluent. A conical flask containing raw textile effluent (25 ml) was inoculated with fungal culture (5%, v/v), followed by incubation at 35°C, 100 rpm (Rajhans et al. 2020). At regular intervals, aliquots were obtained from the flask and then centrifuged (10,000 × g) for 10 min. Thereafter, the conventional indicators for raw and treated effluent such as, COD
(Chemical Oxygen Demand), BOD (Biological Oxygen Demand), color and concentration were assessed following the Standards Methods (APHA 2017). COD concentrations were measured using the potassium dichromate method; Pt-Co color scale was used to measure color. Dye concentration of the sample was determined using colorimeter (Systonic, S-912). Every experiment has been carried out in triplicates and standard deviation has been presented with the average data.

The following equation (Eq. (1)) was used to quantify degradation as a percentage reduction of COD:

\[
\text{%COD reduction} = \frac{\text{COD}_{\text{initial}} - \text{COD}_t}{\text{COD}_{\text{initial}}} \times 100 \quad (1)
\]

\text{COD}_{\text{initial}}: \text{initial value of COD}
\text{COD}_t: \text{value of COD at time ‘t’ (h)}

2.4. Growth kinetics

The following equation (2) has been used to determine the specific growth rate of G. candidum.

\[
\ln \frac{x}{x_o} = \mu t \quad (2)
\]

\(x\): biomass concentration (g L\(^{-1}\)) at ‘t’ time
\(x_o\): initial biomass concentration (g L\(^{-1}\)) at ‘t_0’ time
\(\mu\): specific growth rate (h\(^{-1}\)).

The expression for growth yield (Y) is

\[
\frac{dx}{ds} = Y \quad (3)
\]

The equation 3 can be rewritten as follows (Vijayalakshmidevi and Muthukumar 2015):

\[
x - x_o = Y(S_o - S) \quad (4)
\]

2.5. Analytical studies

The metabolites formed in textile effluent after decolorization and degradation were obtained by same volume with ethyl acetate. The extract was dried over anhydrous sodium sulfate and evaporated to dryness in a rotary evaporator. The resulting crystals were dissolved in small volumes of methanol (HPLC grade), and then used for analysis such as FT-IR (Fourier-transform infrared spectroscopy), LCMS (Liquid chromatography–mass spectrometry) and XRD (X-ray Diffraction).

The FTIR analysis of the effluent was performed with Attenuated total reflectance- Fourier transform infrared spectroscopy (ATR-FTIR, FT/IR-4600, JASCO, Japan). A drop from each sample was placed on a Zinc selenide (ZnSe) frame, and the spectra were documented with an average of 32 scans between the 4000 and 600 cm\(^{-1}\) spectral ranges.

The raw and treated samples were also analyzed using LC-MS (Waters Micromass Q-Tof Micro) and the flow rate and temperature were maintained at 0.2 ml min\(^{-1}\) and 35°C, respectively. The running time was 41 minutes. Two
different solvents with varying proportions, such as water with 0.1% formic acid and acetonitrile with 0.1% formic acid were used. The deuterium lamp (DL) temperature was set at 250°C with m/z value 50–1000 runs in the positive ion mode.

X-ray diffraction patterns before and after biodegradation of textile effluent were recorded using RIGAKU-ULTIMA IV, Japan diffractometer with monochromatic CuKα radiation (λ=1.5406) over the range of 10–90° (2θ). The metals were identified with powder diffraction standard file (JCPDS, Joint Committee on Powder Diffraction Standards Newtown Square, Pennsylvania, USA).

2.6. Toxicity study

2.6.1. Phytotoxicity

The phytotoxicity analysis was performed using Phaseolus mungo (at room temperature) on both raw and treated effluent. Simultaneously, the control set was conducted using water. After 7 days, toxicity of the raw and treated effluent was evaluated by the length of radical, plumule and germination percentage. Mean with standard deviation for all results were presented.

2.6.2. Microbial toxicity

A short-term toxicity test of textile effluent before and after treatment was demonstrated by exposing the bacteria Escherichia coli (ATCC 443) to the textile effluent for 15 min. Toxicity to E. coli was determined spectrophotometrically by evaluating the difference in the number of cells before and after treatment. All the tests were conducted in triplicate. The experiment with control set was also carried out.

2.6.3. Genotoxicity Study

The study of genotoxicity was carried out with Allium cepa. Both raw and treated textile effluent is used to treat the roots. The growths after 48h of incubation at room temperature were examined (Jadhav et al. 2010). A light microscope (NikonH600L Eclipses LV100) was used to obtain mean values of root length, mitotic index (MI) and chromosomal aberrations in the cell. The experiment was conducted in triplicates and the mean ± standard deviation values were accounted.

2.6. Statistical analysis

The toxicity test results for E. coli were expressed as EC_{50}, which represented a 50% inhibition of E. coli growth caused by a percentage concentration of the textile effluent (v/v). EC_{50} was evaluated via the linear interpolation method (Norberg-King 1993).

Linear Regression method is to fit concentration– inhibition data to a linear regression and then to calculate EC_{50} values by linear interpolation. Interpolation log method is according to Huber and Koella (1993) using the following formula, Eq.(5) to calculate EC_{50} value.

\[
\log(EC_{50}) = \log(x_1) + \frac{50\% - y_1}{y_2 - y_1} \times [\log(x_2) - \log(x_1)] \tag{5}
\]

Direct Interpolation method is similar to interpolation log method but without logarithmic transformation of concentrations. The equation Eq.(6) for EC_{50} calculation is according to (Alexander et al. 1999) as follows.

\[
EC_{50} = x_1 + \frac{50\% - y_1}{y_2 - y_1}(x_2 - x_1) \tag{6}
\]
x₁, x₂: two conc. of the textile effluent;
y₁, y₂: corresponding inhibitions (y₁ < 50%; y₂ > 50%).

% Inhibition can be calculated from the following formula (7)(Wang et al. 2010)

\[
\frac{A_{\text{control}} - A_{\text{sample}}}{A_{\text{control}}} \times 100
\]  

After evaluation of EC₅₀, the toxicity of all samples were expressed as toxic units, TU (unitless), as per equation (8) (Sprague 1970),

\[
TU_{50} = \frac{100}{EC_{50}}
\]  

The Pearson’s correlation coefficient at the significance level of 0.05 was used to assess the correlation between toxicity and conventional indicators of the raw and treated textile effluent, and the impact of COD on toxicity was analyzed by means of linear regression. The significance level of the regression analysis (p) and R² illustrated the extent of toxicity variance caused by COD.

3. Results and Discussion

3.1. Conventional indicators of textile effluent

The conventional indicators of raw and treated textile effluent have been listed in Table 1. It was evident that the raw textile effluent was high in COD and BOD concentrations (Yaseen and Scholz 2016), which was way much higher than the permitted levels (COD - less than 250 mg/L and BOD - less than 30 mg/L in India). Studies show that common components of dye effluents such as some acid dyes and ionic dyes could easily escape into the environment leading to elevating levels of COD, BOD and coloration of water bodies (Lee et al. 2013). After treatment, it was observed that G. candidum was able to remove COD (98.5%), BOD (96.3%), concentration (87%), color (89%) from the textile effluent in 18 h, which meets the discharge standards. The high removal efficiency of G. candidum improved the quality of textile effluent in terms of COD, BOD and color. A recent study showed the reduction in COD, BOD and color of the textile effluent by 77.5%, 71.0 % and 99.2% respectively in 24 hours after treatment with Aspergillus niger (Gurbuz et al. 2019). Yet another study showed that 91%, 88% and 68% reduction was recorded in the color intensity, COD and BOD of the textile wastewater, respectively, after treatment with Peyronellaea prosopidis (Bankole et al. 2018).

Table 1. Conventional indicators in raw and treated textile effluent

| Indicators            | Raw textile effluent | Treated textile effluent (after 18h) | Removal (%) (after 18h) |
|-----------------------|----------------------|-------------------------------------|-------------------------|
| COD (mg/L)            | 902.1 ± 0.05         | 13.20 ± 0.05                       | 98.5                    |
| BOD (mg/L)            | 180.42 ± 0.02        | 6.64 ± 0.02                        | 96.3                    |
| Color (hazen units)   | 35400                | 3894                                | 89                      |
| Concentration of sample(ppm) | 3100                | 400                                 | 87                      |

3.2. Growth Kinetics Studies

The specific growth rate of 0.127 h⁻¹ and yield coefficient of 2.64 mg of dry weight of biomass/mg COD were obtained for the biodegradation using G. candidum. This indicated that G. candidum was able to thrive in the
extreme conditions of the effluent, reducing COD efficiently. The literatures reported the specific growth rate of 0.116 h\(^{-1}\) and yield coefficient of 1.22 mg of dry weight of biomass/mg COD (Vijayalakshmidevi and Muthukumar 2015). Yet another report showed the specific growth rate varying from 0.001 to 0.003 h\(^{-1}\) and yield coefficient varying from 0.25 to 0.75 mg of dry weight of biomass/mg COD (Bayram et al. 2017).

### 3.3. Characterization of biodegraded textile effluent

**FT-IR:** *G. candidum*-induced effluent biodegradation was established through FTIR spectral analyses (Fig. 1). The process of biodegradation is demonstrated either by loss of absorbance peaks or by the occurrence of new peaks (Chen et al. 2003; Sen et al. 2019). The FTIR spectrum of the untreated effluent represented the variable stretching vibrations of C=C (alkenyl), C=C (alkyne), P-H (phosphine) and N-H(aamine) at 1635 cm\(^{-1}\), 2127 cm\(^{-1}\), 2360 cm\(^{-1}\) and 3348 cm\(^{-1}\) respectively. The biodegradation products obtained after 18 h of treatment showed disappearance of phosphine group and occurrence of a new peak at 530 cm\(^{-1}\), representing the occurrence of strong stretching vibration of C-Br (alkyl bromide)(Fig. 1). The biodegradation of raw textile effluent by *G. candidum* was clearly established in this study. Previous studies have established that the biodegradation of textile dyes can be confirmed by FT-IR spectrum representing occurrence of new peaks (Sen et al. 2019).

![FT-IR spectral analyses of raw and treated textile effluent](image)

**Fig.1** FT-IR spectral analyses of raw and treated textile effluent

**LC-MS:** In positive ion and full scanning mode, the textile dye effluent was analyzed from 100 to 1000 m/z. Several mass peaks of varying values have been observed with both raw and treated effluent (Fig. 2a). For the raw effluent, a mass peak at 453 m/z (m.w. 452) was identical to that of an azo dye, acid orange 10 (Fig. 2b). Hence, it was evident that the acid orange 10 was a major dye component of the textile effluent used in this study. The identification of metabolites produced prior to biodegradation of effluent was carried out and the plausible biodegradation pathway based on the previous reports was predicted. (Chacko and Subramaniam 2011). The secretion and involvement of ligninolytic enzymes (laccase and lignin peroxidase) in the azo dye degradation process of *G. candidum* was evident from our previous study (Rajhans et al. 2020). The degradation of acid orange 10 by peroxidase leads to the formation of two biodegradation products such as 7-oxo-8-iminonaphthalene-1,3-disulfonate...
and nitrosobenzene, which subsequently undergoes stepwise hydrogenation and dehydration to form aniline (m.w. 93, m/z 91) (Fig. 2c), via phenyl hydroxyl amine intermediate (as proposed by Mahata and co-workers (2014)). The degradation pathway has been predicted in Fig. 3. Aniline was found in the treated effluent as a low molecular weight compound, rendering it less toxic.
XRD: Heavy metal ions can be prevalent in textile effluent because of the metal-associated dyes and/or additional components used in the dyeing process. The X-ray spectra obtained for the dried samples of the untreated and treated effluent after 18 h treatment with G. candidum are shown in Fig. 4. This displayed various peaks that specifically indicated the presence of metals in raw effluent. The 2θ values of 32.36° and 50.5° showing the presence of major metals, such as lead and mercury respectively. The presence of these metals was confirmed using standard JCPDS reference code (04-0686(Pb) and 01-085-0211(Hg)). Subsequently, the X-ray spectra of treated effluent showed absence of Pb as well as the considerable decrease in the peak intensity for Hg indicating the decreased toxicity of the effluent (Fig. 4). A decrease in % crystallinity was observed in the textile effluent after it was exposed to G. candidum, indicating the degradation of the effluent. Previous studies have demonstrated similar removal of the heavy metals from textile effluent (Vijayalakshmi and Muthukumar 2015). The presence of heavy metals in the effluent lead to numerous health hazards and sequential treatment would eliminate the heavy metals, and make the treated effluent more safe to discharge into the environment.

3.4. Toxicity evaluation

3.4.1. Phytotoxicity

Agriculturally valuable seeds i.e. P. mungo were used for phytotoxicity assessment of raw and treated textile effluent. The analyzed parameters were germination percentage, plumule and radical length. The seeds treated with water were considered as positive control and the test samples were significantly compared with each other, a maximum germination of 100% was recorded in P. mungo with treated textile effluent indicating it as less phytotoxic. Seeds treated with raw textile effluent showed a minimal germination of 33% (Fig. 5). Though the seeds exposed to raw textile effluent germinated, they couldn’t grow further, exhibiting maximum phytotoxicity. The results shown in Table 2 indicated that the germination (%) and length of plumule and radicle of P. mungo seeds were less with the untreated as compared to treated effluent. This study shows that the metabolites formed after effluent biodegradation are less harmful than the compound present in the raw textile effluent. It is evident that the seed germination and average plumula and radical development were unaffected by decolored textile effluent. Thus, the comprehensive results indicated that the textile effluent treated with G. candidum was not harmful to plant germination and growth. Similar kind of phytotoxicity studies can be seen reported for several times in the literature. This ensures that treated effluent could be used for agriculture or recycled.
Fig. 5 Phytotoxicity analysis of (a) Control (Tap water), (b) Treated textile effluent, (c) Raw textile effluent on *P. mungo*.

Table 2 Phytotoxicity study of untreated and treated textile effluent on *P. mungo*

| Parameters          | Control       | Raw textile effluent | Treated textile effluent |
|---------------------|---------------|----------------------|--------------------------|
| Germination (%)     | 98 ± 0.08     | 33 ± 0.06            | 95 ± 0.03                |
| Plumule (cm) Mean ± SD | 6.86±0.954    | 0.28±0.577           | 0.6±0.441                |
| Radicle (cm) Mean ± SD | 8.38±2.399    | 3.15±1.755           | 6.46±1.503               |

Values are mean of three experiments, SD(n), significantly different from the control (seeds germinated in water) at p > 0.05 (One-way analysis of variance, ANOVA).

3.4.2. Toxicity test with *E. coli*

The short-term toxicity of the raw and treated textile effluent was assessed using the well-established bacteria *E. coli* and the results are shown in Fig. 6. The raw textile effluent was highly toxic, while treated textile effluent presented a low toxicity. Fig. 6 shows that after 30 minutes of exposure to raw textile effluent, the number of bacterial cells declined drastically by approximately 41%, indicating acute toxicity to *E. coli*. This was plausibly due to the enormous amount of ionic and acid dyes entering the wastewater during the textile processing. Ionic and disperse dyes discharged from textile processing and dyeing were usually particularly toxic, and some were mutagenic and carcinogenic (Carneiro et al. 2010; Li et al. 2014). On the contrary, it was found that the treated effluent was absolutely harmless to the bacteria, which was evident from their uninhibited growth (cells/ml increased by approximately 10%). This indicates that the toxicity of textile effluent was reduced to a greater extent after treatment with *G. candidum*.
3.4.3. Genotoxicity analysis

The A. cepa study is a standard test to assess the genotoxicity of any toxic substance. The test was carried out to identify MI and chromosomal aberrations in the root cells (Table 3). On the basis of the MI value, (MI value can act as a biosensor for environmental contaminants) the cytotoxic effect of the toxic compound was assessed (Caritá and Marin-Morales 2008). Table 3 shows the genotoxic aspects of the textile effluent before and after treatment. The decreased MI value is indicative of decreased cytotoxicity of treated effluent. The textile wastewater typically has a detrimental impact on chromosomal cell division, and this type of aberrations in mitotic cell division is triggered by spindle apparatus proteins malfunctioning (Jadha et al. 2010) or probably due to decrease in ATP synthesis during cell division. The significant reduction in COD level could then contribute to the decline in the number of aberrant mitotic cells after treatment. The findings recorded were similar to the literature data (Caritá and Marin-Morales 2008; Jadhav et al. 2010).

Table 3 Genotoxicity analysis for the untreated and treated effluent

| Analysis   | Raw Effluent   | Treated effluent |
|------------|----------------|------------------|
| RL (cm)    | 3.28 ± 0.65    | 5.84 ± 0.41      |
| MI         | 0.3 ± 1.32     | 0.9 ± 0.562      |
| MN         | Not found      | Not found        |
| CB         | 3              | 1                |
| TA         | 3              | 1                |
| TCA        | 50             | 50               |
| Frequency of TA | 0.5 ± 0.04*   | 0.25 ± 0.005     |

RL: root length; MI: Mitotic index; MN: micronuclei; CB: Chromosome breaks; TCA: total no. of cells analysed; TA: Total no. of alterations. Values are mean of three experiments, SD( _), significantly different from the control (roots germinated in water). "P < 0.05, **P < 0.001 by one-way analysis of variance (ANOVA) with Tukey*Kramer comparison test.

The decrease in colour, COD and BOD might have led to the minimization in the toxicity of textile effluent. This study indicates that the metabolites produced after biodegradation are less toxic than the compounds present in raw effluent.
3.4.4. Relationship between toxicity and COD of raw and treated textile effluent

Pearson’s correlation analysis for the textile effluent indicated a significantly positive correlation between COD and TU$_{50}$ ($r=0.920$, $p < 0.05$, $R^2=0.84$), which suggested that compounds present in the textile effluent were toxic for *E. coli*. However, there was a negative relationship between COD and TU$_{50}$ ($r=0.088$, $p=0.048$, $R^2=0.77$) in case of treated textile effluent. Similar toxicity and COD correlation studies for textile effluent have been carried out with *Vibrio fischeri* and *Desmodesmus subspicatus*. They showed that there was significantly positive correlation between *V. fischeri* and COD; color and TU$_{50}$ ($r=0.824$, $0.57$, $p < 0.05$), which suggested that compounds producing color might be toxic for *V. fischeri*. However, there was a negative relationship between *D. subspicatus* and TU$_{50}$ ($r=0.625$, $p=0.035$) (Liang et al. 2018).

COD was one of the most widely used water quality monitoring metrics and also was an important measure for the regulation of the usage of wastewater treatment facilities, taxation and surveillance of wastewater effluent (Zheng et al. 2008). Therefore, it was important to establish associations between bio-toxicity and conventional markers such as COD (Raptis et al. 2014).

Conclusion

The competent *Geotrichum candidum* culture involved in the current work biodegraded the toxic textile effluent. The analysis of conventional parameters such as COD, BOD and color were indicative of the decreased toxicity of the treated effluent in comparison to the raw effluent. The effective decolorization and biodegradation of effluent in 18h was confirmed by FT-IR and XRD analysis. The plausible biodegradation pathway has been proposed on the basis of metabolites identified by LCMS. This demonstrated the first report on the proposed pathway for enzymatic degradation study of acid orange 10 by *G. candidum*. The genotoxicity, phytotoxicity and microbial toxicity analyses proved the raw effluent is harmful, whereas the treated effluent is less toxic. Relationship between effluent COD and TU$_{50}$ showed that an increase in effluent COD resulted in increase in wastewater toxicity. There was a clearly defined correlation between toxicity and COD. It was evident that toxic effects of the textile effluent were significantly reduced upon treatment with *G. candidum*. The major relationships between toxicity and COD will provide directions for more efficient control of textile dyeing effluents. This is the first report on the positive correlation between toxicity and COD of textile effluent using *G. candidum* within a very short stretch of time (18h). Therefore, the findings of this study have shown that the treated effluent was safer to be released in regard to physicochemical parameters and toxicity unit (TU$_{50}$). The correlation between conventional indicators and toxicity may provide assistance in effluent management.

References

Agrawal K, Verma P (2019) Biodegradation of synthetic dye Alizarin Cyanine Green by yellow laccase producing strain *Stropharia* sp. ITCC-8422. Biocatal Agric Biotechnol 21:101291. https://doi.org/10.1016/j.bcab.2019.101291

Alexander B, Browne DJ, Reading SJ, Benjamin IS (1999) A simple and accurate mathematical method for calculation of the EC50. J Pharmacol Toxicol Methods 41:55–58. https://doi.org/10.1016/S1056-8719(98)00038-0

Bankole PO, Adekunle AA, Obidi OF, et al (2018) Biodegradation and detoxification of Scarlet RR dye by a newly isolated filamentous fungus, *Peyronellaea prosopidis*. Sustain Environ Res 28:214–222. https://doi.org/10.1016/j.serj.2018.03.001

Bayram TT, Nuhoğlu A, Aladağ E (2017) Investigation of biodegradation and growth kinetics of dairy wastewater in a batch reactor. Bulg Chem Commun 49:896 – 900

Caritá R, Marin-Morales MA (2008) Induction of chromosome aberrations in the Allium cepa test system caused by the exposure of seeds to industrial effluents contaminated with azo dyes. Chemosphere 72:722–725.
Mahata A, Rai RK, Choudhuri I, et al (2014) Direct vs. indirect pathway for nitrobenzene reduction reaction on a Ni catalyst surface: a density functional study. Phys Chem Chem Phys 16:26365–26374.
Wang J, Liu H, Zhao J, et al (2010) Antimicrobial and Antioxidant Activities of the Root Bark Essential Oil of Periploca sepium and Its Main Component 2-Hydroxy-4-methoxybenzaldehyde. Molecules 15:5807–5817. https://doi.org/10.3390/molecules15085807

Yaseen DA, Scholz M (2016) Shallow pond systems planted with Lemna minor treating azo dyes. Ecol Eng 94:295–305. https://doi.org/10.1016/j.ecoleng.2016.05.081

Yin, L., Chen, Z.-H & Zhao S-J (2008) Optimization of laccase production from Geotrichum candidum and decoloration of azo dyes by laccase. 36:85–90

Yu, W., Liu, W., Huang, H., Zheng, F., Wang, X., Wu, Y., … Jin Y (2014) Application of a Novel Alkali-Tolerant Thermostable DyP-Type Peroxidase from Saccharomonospora viridis DSM 43017 in Biobleaching of Eucalyptus Kraft Pulp. PLoS One 9:110319. https://doi.org/10.1371/journal.pone.0110319

Zheng Q, Zhou B, Bai J, et al (2008) Self-Organized TiO2 Nanotube Array Sensor for the Determination of Chemical Oxygen Demand. Adv Mater 20:1044–1049. https://doi.org/10.1002/adma.200701619