Mycoremediation of Textile Effluent: A Toxicological Evaluation and its Possible Correlation with COD

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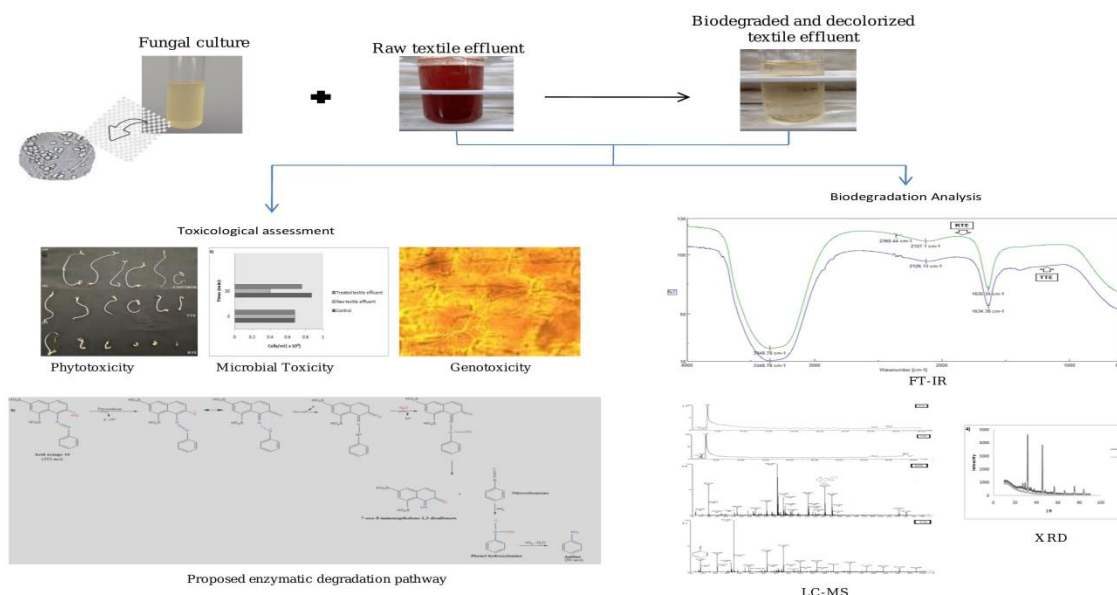6 *[c]Department of Chemistry, ITER, Siksha O Anusandhan (Deemed to be University), Bhubaneswar -751003, Odisha,*
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12

13 **Abstract**

14

15 Globally, textile industries are one of the major sectors releasing dye pollutants. This is the first report on the
16 positive correlation between toxicity and COD of textile effluent along with the proposed pathway for enzymatic
17 degradation of acid orange 10 using *Geotrichum candidum* within a very short stretch of time (18h). Removal
18 efficiency of this mycoremedial approach after 18 h in terms of color, dye concentration as well as reduction of
19 chemical oxygen demand (COD) and biological oxygen demand (BOD) in the treated effluent reached to 89%, 87%,
20 98.5% and 96.3% respectively. FT-IR analysis of the treated effluent confirmed biodegradation. The LC-MS
21 analysis showed the degradation of acid orange 10, which was confirmed by the formation of two biodegradation
22 products, 7-oxo-8-iminonapthalene-1,3-disulfonate and nitrosobenzene, which subsequently undergoes stepwise
23 hydrogenation and dehydration to form aniline via phenyl hydroxyl amine as intermediate. The X-ray diffraction
24 (XRD) studies showed that heavy metals content in the treated effluent has reduced along with decrease in %
25 crystallinity, indicating biodegradation. The connection between toxicity and COD was also inveterated using
26 Pearson's correlation coefficient. Further the toxicological studies indicated the toxicity of raw textile effluent and
27 relatively lower toxic nature of metabolites generated after biodegradation by *G. candidum*.

28
29 **Graphical Abstract**

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54
55

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

67 recalcitrants in untreated effluents are both harmful to marine and terrestrial organisms and have prolonged effects
68 on health (Hamidi et al., 2014).
69 To assess the performance of wastewater treatment facilities, the influent and treated water samples after each 51
70 treatment phase (physical, chemical and biological) should be tracked. In general, microbial degradation is known to
71 be a safe, natural, inexpensive and effective pollutant removal technique in the world (Mishra et al. 2016; Singh et
72 al. 2017, 2018; Kumar et al. 2017). The eco-friendliness and low cost of biological technology has in particular
73 gained significant attention (Maljaei et al. 2009). However, bacteria typically contribute to the breakdown of textile
74 dyes creating and accumulating more intractable or hazardous aromatic amine substances that restrict their
75 comprehensive applications to azo dye wastewater treatment plants (Davies et al. 2006). Enhanced techniques are
76 evidently the pre-condition for accelerated elimination of azo dyes, because any residual contaminants should be
77 removed completely. Fungi have been investigated, especially those secreting non-specific oxidases which
78 eventually lead to the azo dyes mineralization into CO_2 (Wanderley et al. 2018). Fungal degradation
79 (Mycoremediation) also leads to complete decoloration and detoxification, which prevents sludge removal and
80 secondary contamination issues.

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

84 *Geotrichum* sp. out of the few fungi have been observed for degradation of large quantity of artificial colors and
85 molasses (Kim and Shoda 1998, 1999; Chen and Zhao 2008; Shintani and Shoda 2013). Since, *Geotrichum* sp have
86 not been explored much, therefore it is being used in the current study for the biodegradation and detoxification of
87 textile effluent. The analysis of conventional parameters of textile effluent (before and after mycoremediation) and
88 the inter-relationship between toxicity and COD have been carried out in this study. In an attempt to validate the
89 non-toxicity of treated effluent, the biodegradation analysis such as FT-IR, LC-MS and XRD have been conducted.
90 Furthermore, bioassays such as genotoxicity, phytotoxicity and microbial toxicity assays have also been carried out
91 so that the toxicity level of raw and treated textile effluent can be assessed.
92
93 **2. Materials and Methods**
94
95 **2.1. *Collection of samples***
96
97 Samples of textile dyeing effluent have been obtained from a nearby textile factory in Khurda, India. During regular
98 operations, factory workers sampled 100mL of wastewater every two hours to ensure that the study involves
99 variability in substances.
100
101 **2.2. *Microbial culture conditions***
102
103 This work utilizes *Geotrichum candidum,* a ubiquitous fungus belonging to *Dipodoascaceae* family. It was grown at
104 35 °C on Potato Dextrose Agar plates (PDA) (pH-5.6 ± 0.2). Pure fungal culture was inoculated in 3 percent malt
105 extract broth after 24 hours to maintain the strain and cultured at 35 °C, 100 rpm. Using DP media (dextrose and
106 peptone in ratio of 2:3) at 35 °C and 100 rpm, the optimum fungal growth was achieved (Rajhans et al. 2019).
107
108 **2.3. *Analysis of conventional indicators of textile effluent***
109
110 In this part of the analysis, the *G. candidum* culture was used to biodegrade the textile effluent. A conical flask
111 containing raw textile effluent (25 ml) was inoculated with fungal culture (5%, v/v), followed by incubation at 35°C,
112 100 rpm (Rajhans et al. 2020). At regular intervals, aliquots were obtained from the flask and then centrifuged
113 (10,000 × g) for 10 min. Thereafter, the conventional indicators for raw and treated effluent such as, COD

114 (Chemical Oxygen Demand), BOD (Biological Oxygen Demand), color and concentration were assessed following
115 the Standards Methods (APHA 2017). COD concentrations were measured using the potassium dichromate method;
116 Pt-Co color scale was used to measure color. Dye concentration of the sample was determined using colorimeter
117 (Systonic, S-912). Every experiment has been carried out in triplicates and standard deviation has been presented
118 with the average data.
119 The following equation (Eq. (1)) was used to quantify degradation as a percentage reduction of COD:
120

$$\%COD_{reduction} = \frac{COD_{initial} - COD_t}{COD_{initial}} \times 100 \qquad (1)$$

123 $COD_{initial}$:- initial value of COD
124 COD_t:- value of COD at time 't' (h)

126 **2.4. *Growth kinetics***

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

$$ln\frac{x}{x_o} = \mu t \qquad (2)$$

132 x: biomass concentration (g L^{-1}) at 't' time
133 x_o: initial biomass concentration (g L^{-1}) at 't$_0$' time
134 μ: specific growth rate (h^{-1}).

136 The expression for growth yield (Y) is

$$\frac{dx}{ds} = Y \qquad (3)$$

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

$$x - x_o = Y(S_o - S) \qquad (4)$$

142 S_o: initial substrate (COD) concentration (mg L^{-1})
143 S: final substrate (COD) concentration (mg L^{-1})
144 x: biomass concentration (mg L^{-1})
145 x_o: initial biomass concentration (mg L^{-1})

147 **2.5. *Analytical studies***

149 The metabolites formed in textile effluent after decolorization and degradation were obtained by same volume with
150 ethyl acetate. The extract was dried over anhydrous sodium sulfate and evaporated to dryness in a rotary evaporator.
151 The resulting crystals were dissolved in small volumes of methanol (HPLC grade), and then used for analysis such
152 as FT-IR (Fourier-transform infrared spectroscopy), LCMS (Liquid chromatography–mass spectrometry) and XRD
153 (X-ray Diffraction).
154 The FTIR analysis of the effluent was performed with Attenuated total reflectance- Fourier transform infrared
155 spectroscopy (ATR-FTIR, FT/IR-4600, JASCO, Japan). A drop from each sample was placed on a Zinc selenide
156 (ZnSe) frame, and the spectra were documented with an average of 32 scans between the 4000 and 600 cm^{-1} spectral
157 ranges.

159 The raw and treated samples were also analyzed using LC-MS (Waters Micromass Q-Tof Micro) and the flow rate
160 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.

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

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.

$$EC_{50} = x_1 + \frac{50\% - y_1}{y_2 - y_1}(x_2 - x_1) \qquad (6)$$

207   x_1, x_2: two conc. of the textile effluent;

208   y_1, y_2: corresponding inhibitions ($y_1 < 50\%$; $y_2 > 50\%$).

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

210

211    $$\frac{A_{control} - A_{sample}}{A_{control}} \times 100 \qquad (7)$$

212

213   After evaluation of $EC_{50,}$ the toxicity of all samples were expressed as toxic units, TU (unitless), as per equation (8)

214   (Sprague 1970),

215

216    $$TU_{50} = 100/EC_{50}. \qquad (8)$$

217

218   The Pearson's correlation coefficient at the significance level of 0.05 was used to assess the correlation between

219   toxicity and conventional indicators of the raw and treated textile effluent, and the impact of COD on toxicity was

220   analyzed by means of linear regression. The significance level of the regression analysis (p) and R^2 illustrated the

221   extent of toxicity variance caused by COD.

222

223   **3. Results and Discussion**

224

225   **3.1.** *Conventional indicators of textile effluent*

226

227   The conventional indicators of raw and treated textile effluent have been listed in Table 1. It was evident that the

228   raw textile effluent was high in COD and BOD concentrations (Yaseen and Scholz 2016), which was way much

229   higher than the permitted levels (COD- less than 250 mg/L and BOD- less than 30 mg/L in India). Studies show that

230   common components of dye effluents such as some acid dyes and ionic dyes could easily escape into the

231   environment leading to elevating levels of COD, BOD and coloration of water bodies(Lee et al. 2013). After

232   treatment, it was observed that *G. candidum* was able to remove COD (98.5 %), BOD (96.3%), concentration

233   (87%), color (89%) from the textile effluent in 18 h, which meets the discharge standards. The high removal

234   efficiency of *G. candidum* improved the quality of textile effluent in terms of COD, BOD and color. A recent study

235   showed the reduction in COD, BOD and color of the textile effluent by 77.5%, 71.0 % and 99.2% respectively in 24

236   hours after treatment with *Aspergillus niger* (Gurbuz et al. 2019). Yet another study showed that 91%, 88% and 68%

237   reduction was recorded in the color intensity, COD and BOD of the textile wastewater, respectively, after treatment

238   with *Peyronellaea prosopidis* (Bankole et al. 2018).

Table1. 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**

239

240   **3.2.** *Growth Kinetics Studies*

241

242   The specific growth rate of 0.127 h^{-1} and yield coefficient of 2.64 mg of dry weight of biomass/mg COD were

243   obtained for the biodegradation using *G. candidum*. This indicated that *G. candidum* was able to thrive in the

244 extreme conditions of the effluent, reducing COD efficiently. The literatures reported the specific growth rate of
245 0.116 h–1 and yield coefficient of 1.22 mg of dry weight of biomass/mg COD (Vijayalakshmidevi and Muthukumar
246 2015). Yet another report showed the specific growth rate varying from 0.001 to 0.003 h^{-1} and yield coefficient
247 varying from 0.25 to 0.75 mg of dry weight of biomass/mg COD (Bayram et al. 2017).

248
249 **3.3.** *Characterization of biodegraded textile effluent*

250
251 ***FT-IR***: *G. candidum*-induced effluent biodegradation was established through FTIR spectral analyses (Fig. 1). The
252 process of biodegradation is demonstrated either by loss of absorbance peaks or by the occurrence of new peaks
253 (Chen et al. 2003; Sen et al. 2019). The FTIR spectrum of the untreated effluent represented the variable stretching
254 vibrations of C=C (alkenyl), C=C (alkyne), P-H (phosphine) and N-H(amine) at 1635 cm^{-1}, 2127 cm^{-1}, 2360 cm^{-1}
255 and 3348 cm^{-1} respectively. The biodegradation products obtained after 18 h of treatment showed disappearance of
256 phosphine group and occurrence of a new peak at 530 cm^{-1}, representing the occurrence of strong stretching
257 vibration of C-Br (alkyl bromide)(Fig. 1). The biodegradation of raw textile effluent by *G. candidum* was clearly
258 established in this study. Previous studies have established that the biodegradation of textile dyes can be confirmed
259 by FT-IR spectrum representing occurence of new peaks (Sen et al. 2019).
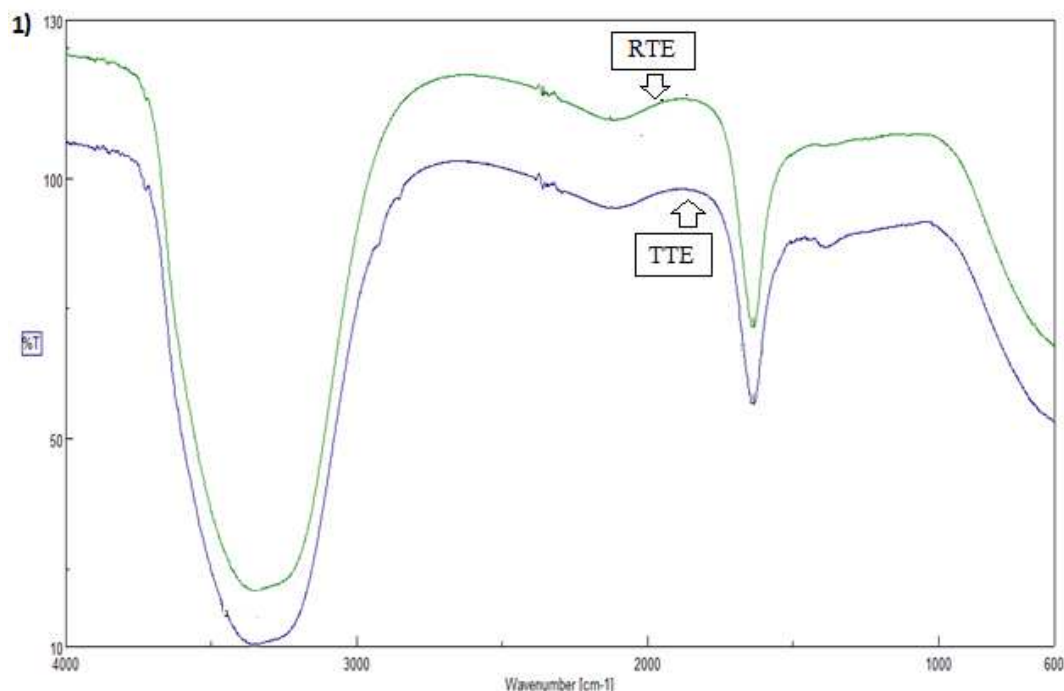

260
261 **Fig.1** FT-IR spectral analyses of raw and treated textile effluent

262
263 ***LC-MS***: In positive ion and full scanning mode, the textile dye effluent was analyzed from 100 to 1000 m/z. Several
264 mass peaks of varying values have been observed with both raw and treated effluent (Fig. 2a). For the raw effluent, a
265 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
266 that the acid orange 10 was a major dye component of the textile effluent used in this study. The identification
267 of metabolites produced prior to biodegradation of effluent was carried out and the plausible biodegradation
268 pathway based on the previous reports was predicted. (Chacko and Subramaniam 2011). The secretion and
269 involvement of ligninolytic enzymes (laccase and lignin peroxidase) in the azo dye degradation process of *G.*
270 *candidum* was evident from our previous study (Rajhans et al. 2020). The degradation of acid orange 10 by
271 peroxidase leads to the formation of two biodegradation products such as 7-oxo-8-iminonapthalene-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.
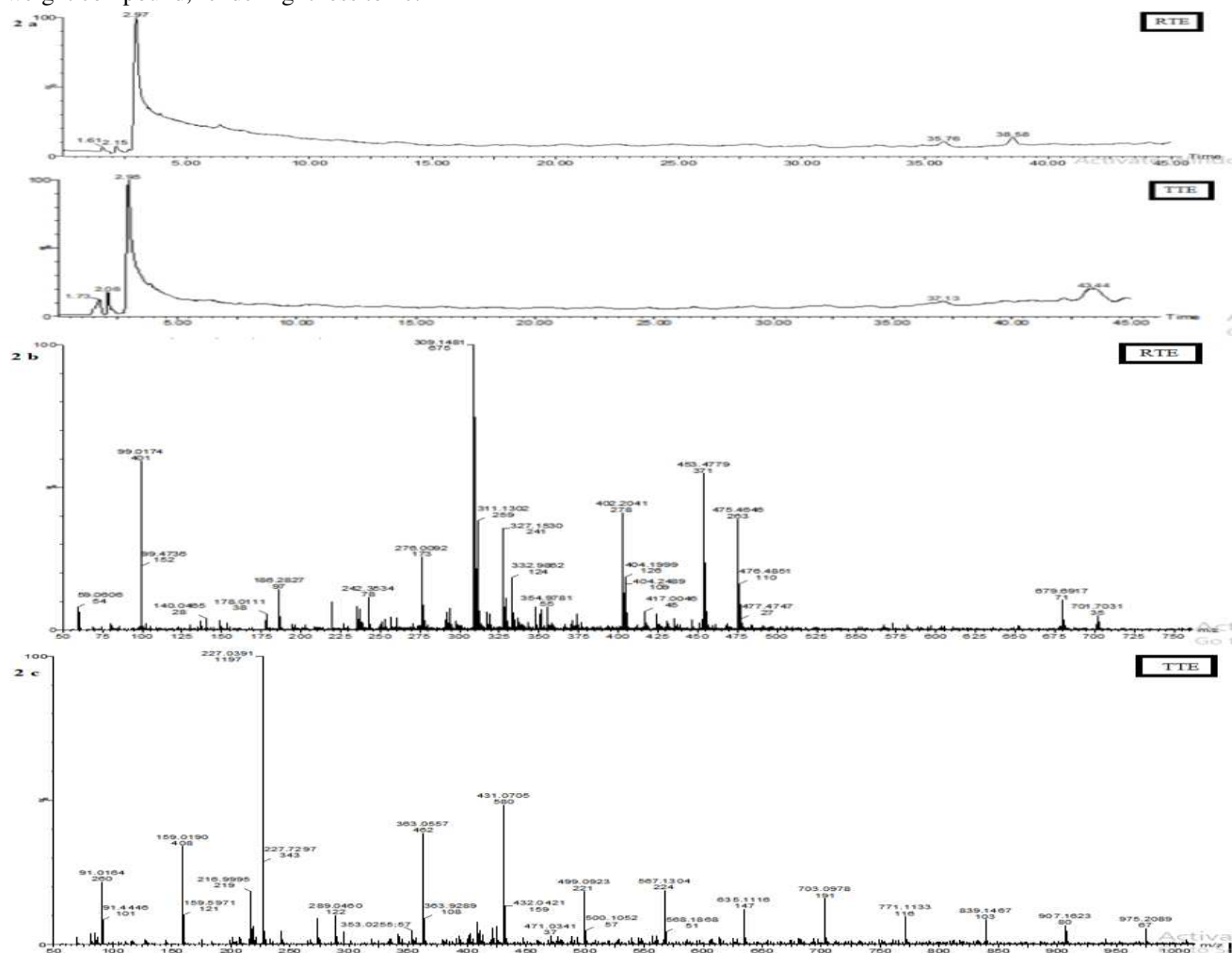

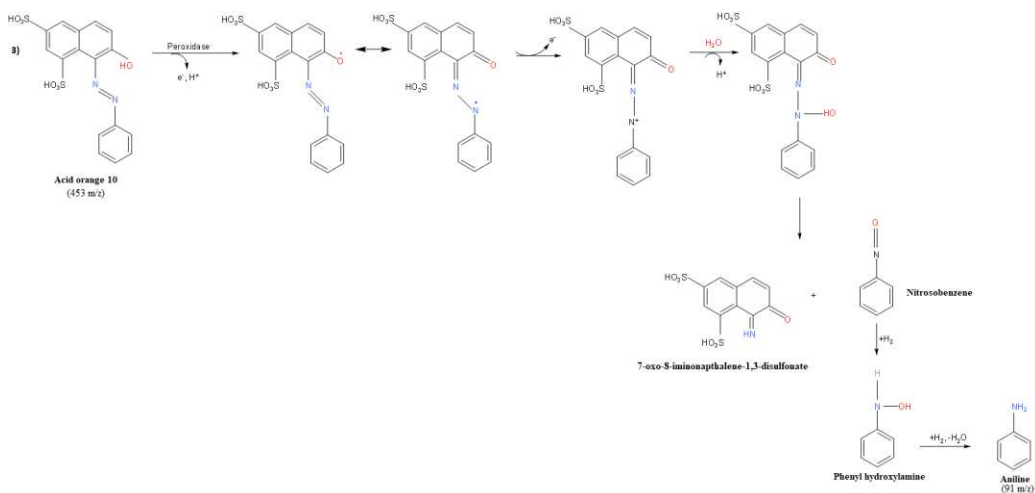**Fig.2** LC-MS analysis of raw and treated textile effluent

8

279   **Fig.3** Proposed pathway for degradation of acid orange 10 dye by *G. candidum*

280
281

282   **XRD**: Heavy metal ions can be prevalent in textile effluent because of the metal-associated dyes and/or additional
283   components used in the dyeing process. The X-ray spectra obtained for the dried samples of the untreated and
284   treated effluent after 18 h treatment with *G. candidum* are shown in Fig. 4. This displayed various peaks that
285   specifically indicated the presence of metals in raw effluent. The 2θ values of 32.36° and 50.5° showing the
286   presence of major metals, such as lead and mercury respectively. The presence of these metals was confirmed using
287   standard JCPDS reference code (04-0686(Pb) and 01-085-0211(Hg)). Subsequently, the X-ray spectra of treated
288   effluent showed absence of Pb as well as the considerable decrease in the peak intensity for Hg indicating the
289   decreased toxicity of the effluent (Fig. 4). A decrease in % crystallinity was observed in the textile effluent after it
290   was exposed to *G. candidum*, indicating the degradation of the effluent. Previous studies have demonstrated similar
291   removal of the heavy metals from textile effluent (Vijayalakshmidevi and Muthukumar 2015). The presence of
292   heavy metals in the effluent lead to numerous health hazards and sequential treatment would eliminate the heavy
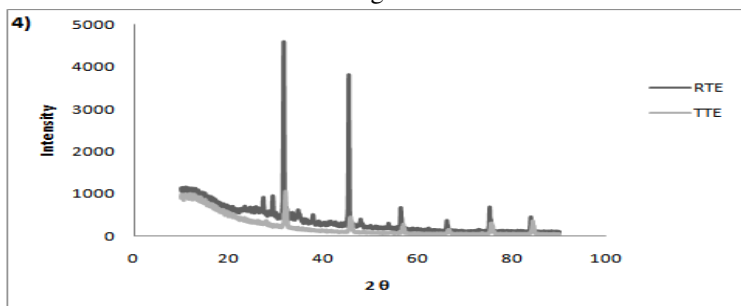293   metals, and make the treated effluent more safe to discharge into the environment.

294
295   **Fig.4** X-ray diffraction pattern of raw and treated textile effluent

296
297   **3.4. Toxicity evaluation**

298

299   **3.4.1.** *Phytotoxicity*

300

301   Agriculturally valuable seeds i.e. *P. mungo* were used for phytotoxicity assessment of raw and treated textile
302   effluent. The analyzed parameters were germination percentage, plumule and radical length. The seeds treated with
303   water were considered as positive control and the test samples were significantly compared with each other, a
304   maximum germination of 100% was recorded in *P. mungo* with treated textile effluent indicating it as less
305   phytotoxic. Seeds treated with raw textile effluent showed a minimal germination of 33% (Fig. 5). Though the seeds
306   exposed to raw textile effluent germinated, they couldn't grow further, exhibiting maximum phytotoxicity. The
307   results shown in Table 2 indicated that the germination (%) and length of plumule and radicle of *P. mungo* seeds
308   were less with the untreated as compared to treated effluent. This study shows that the metabolites formed after
309   effluent biodegradation are less harmful than the compound present in the raw textile effluent. It is evident that the
310   seed germination and average plumula and radical development were unaffected by decolored textile effluent. Thus,
311   the comprehensive results indicated that the textile effluent treated with *G. candidum* was not harmful to plant
312   germination and growth Similar kind of phytotoxicity studies can be seen reported for several times in the literature.
313   This ensures that treated effluent could be used for agriculture or recycled.
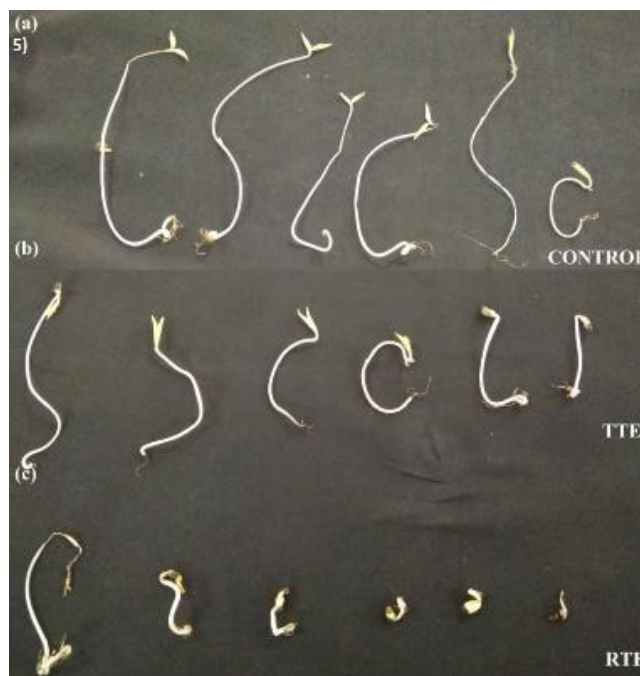
314

315

316   **Fig.5** Phytotoxicity analysis of (a) Control (Tap water), (b) Treated textile effluent, (c) Raw textile effluent on *P.*
317   *mungo*

318   **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

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

320

321   ### 3.4.2. *Toxicity test with E. coli*

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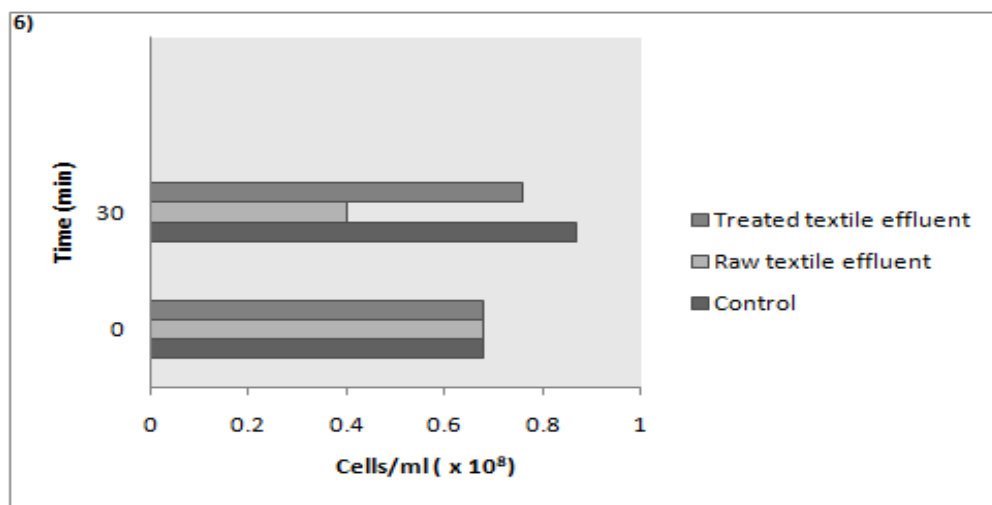331   with *G. candidum*.

Fig.6 Microbial toxicity analysis of (a) Control (culture medium), (b) Treated textile effluent, (c) Raw textile effluent on *E. coli*

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 (Jadhav 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 TukeyeKramer comparison test.

The decrease in colour, COD and BOD might have lead 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.

11

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.

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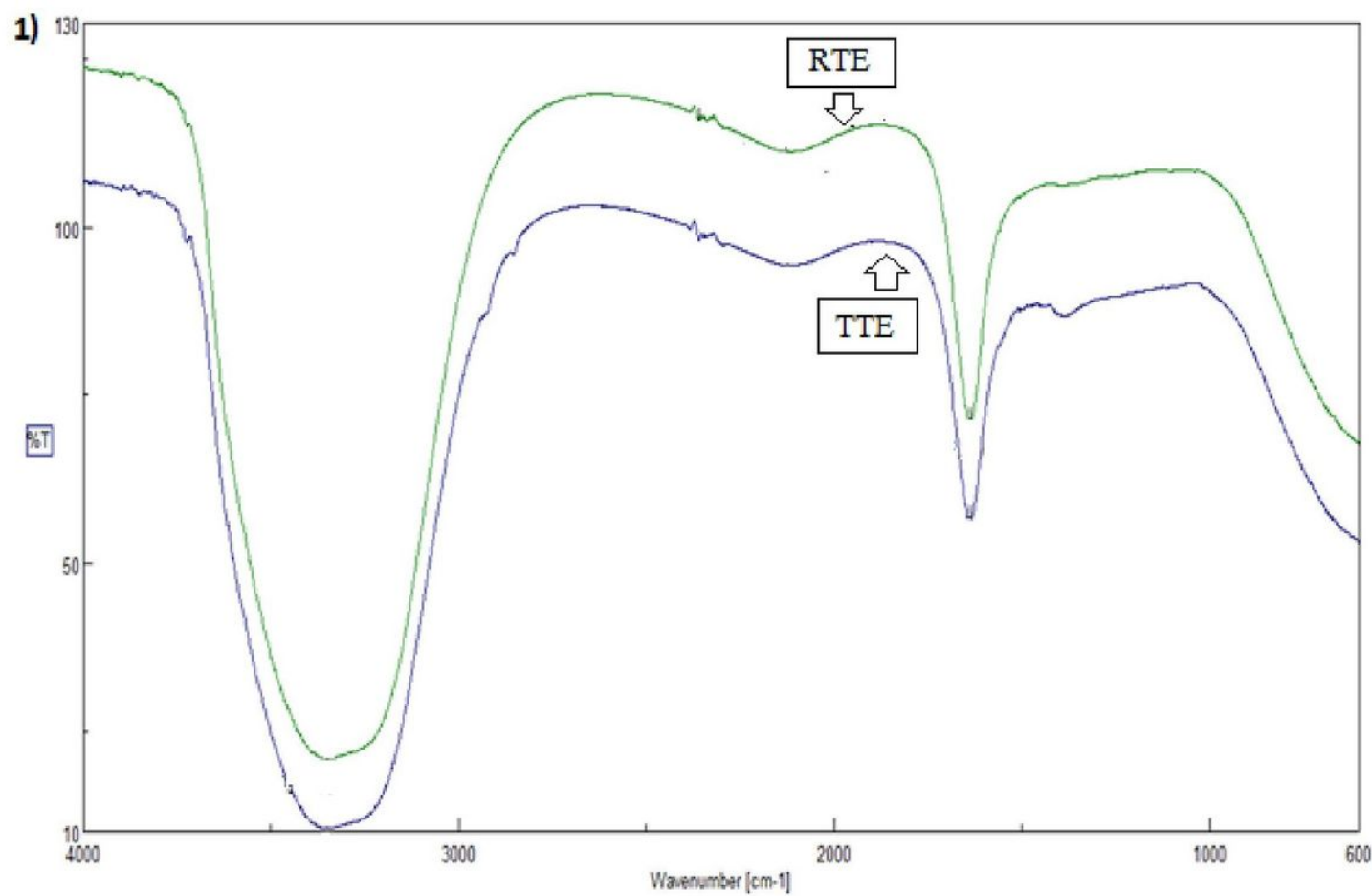518

Figures

Figure 1

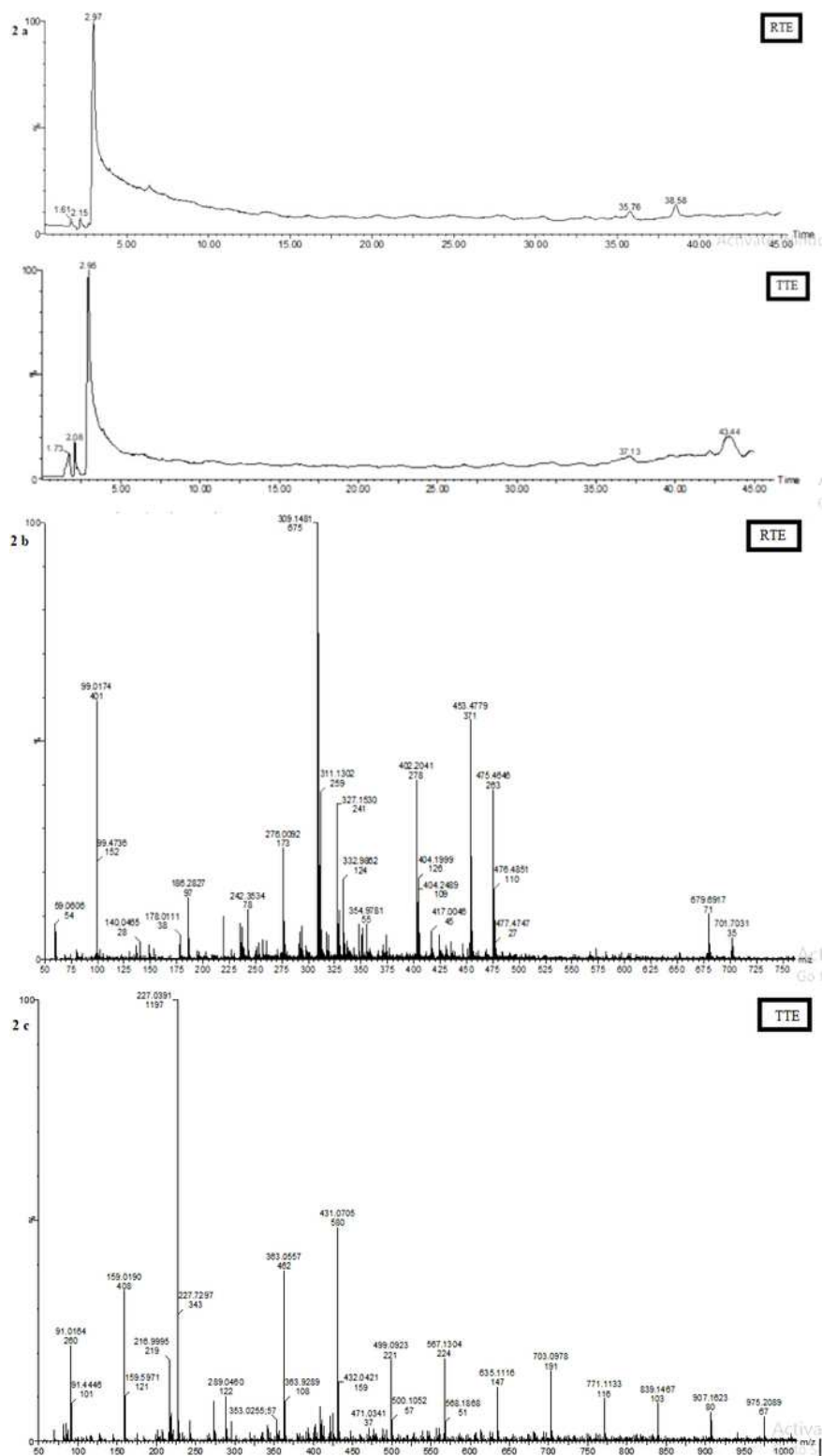FT-IR spectral analyses of raw and treated textile effluent

Figure 2

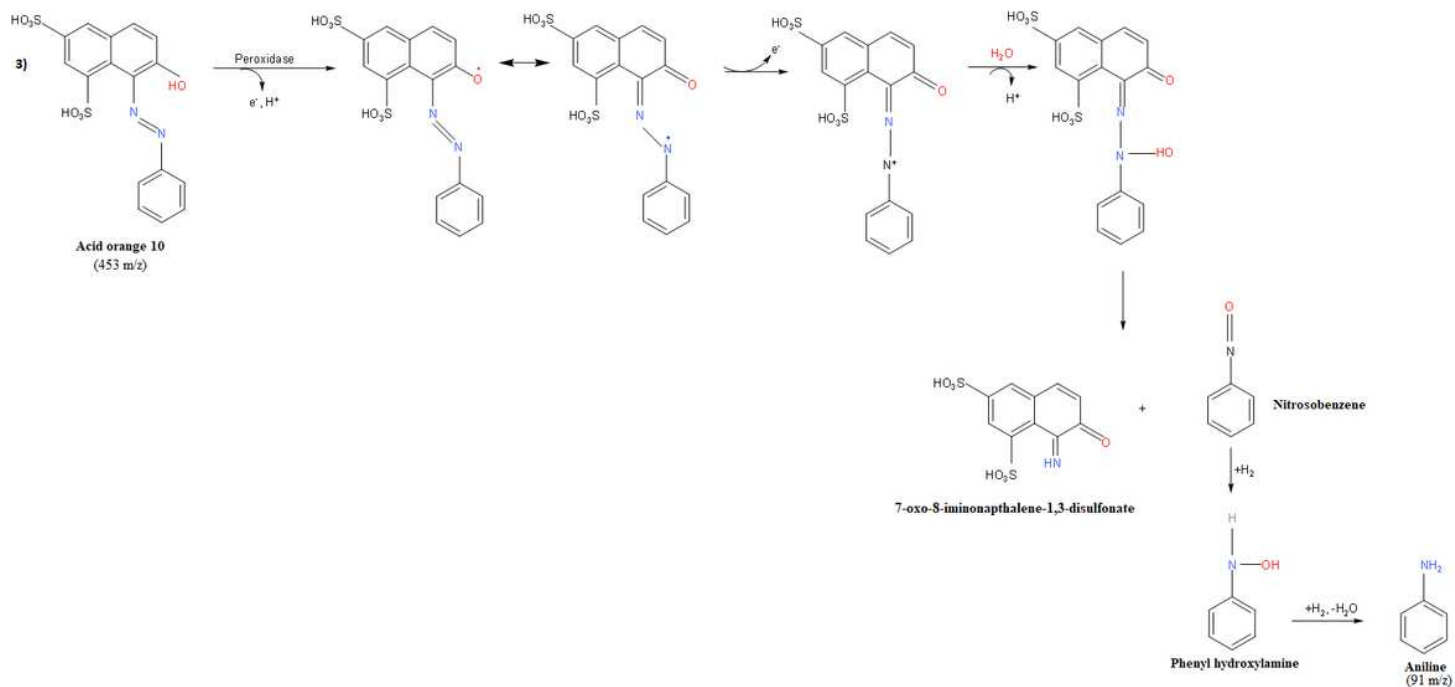LC-MS analysis of raw and treated textile effluent

Figure 3

Proposed pathway for degradation of acid orange 10 dye by G. candidum
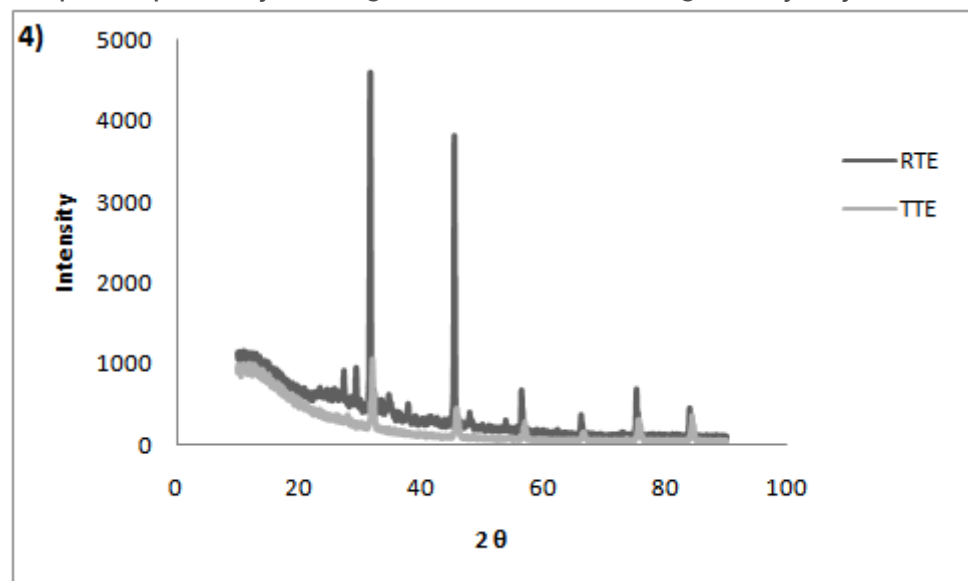

Figure 4

X-ray diffraction pattern of raw and treated textile effluent
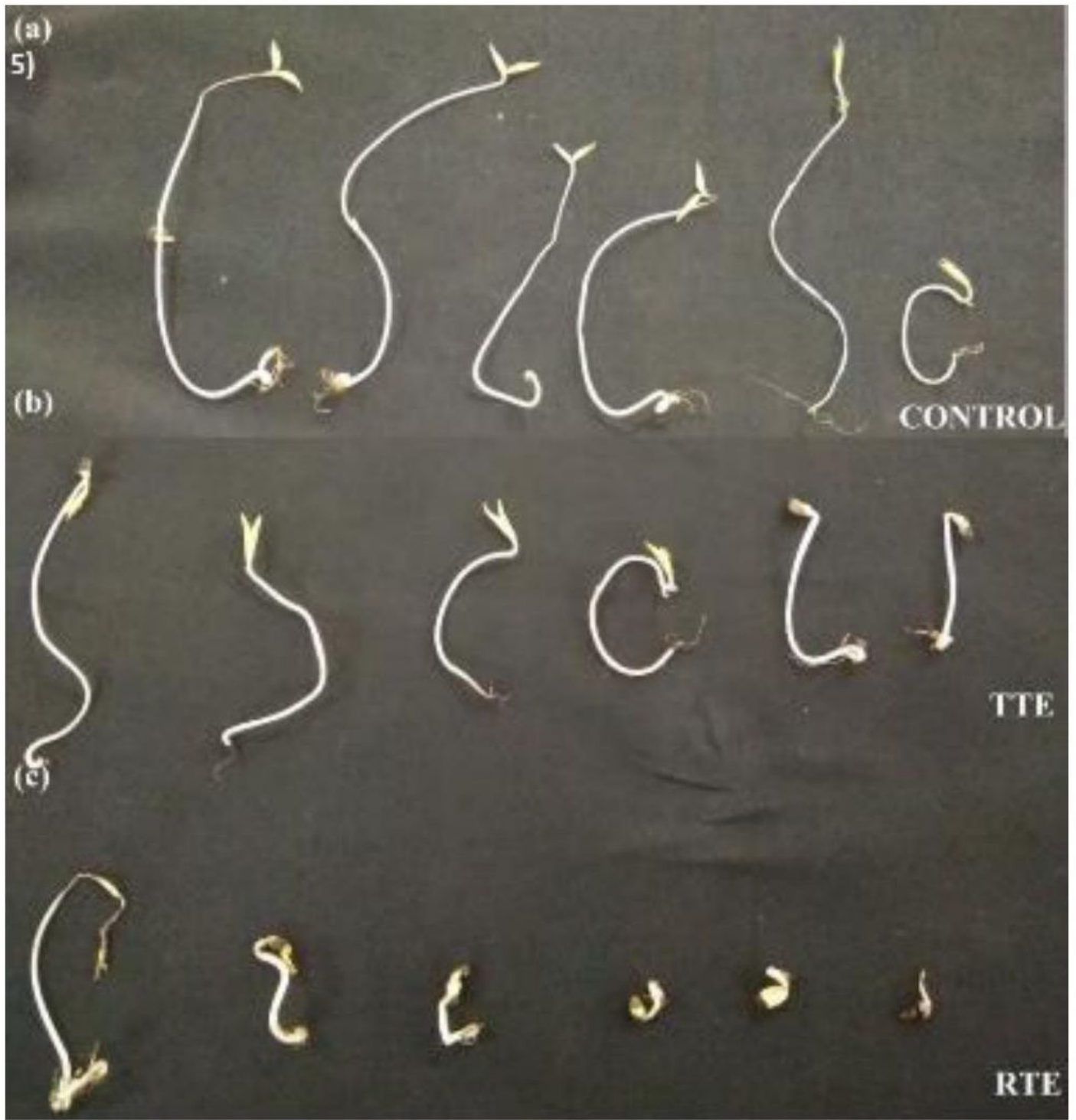
Figure 5

Phytotoxicity analysis of (a) Control (Tap water), (b) Treated textile effluent, (c) Raw textile effluent on P. mungo
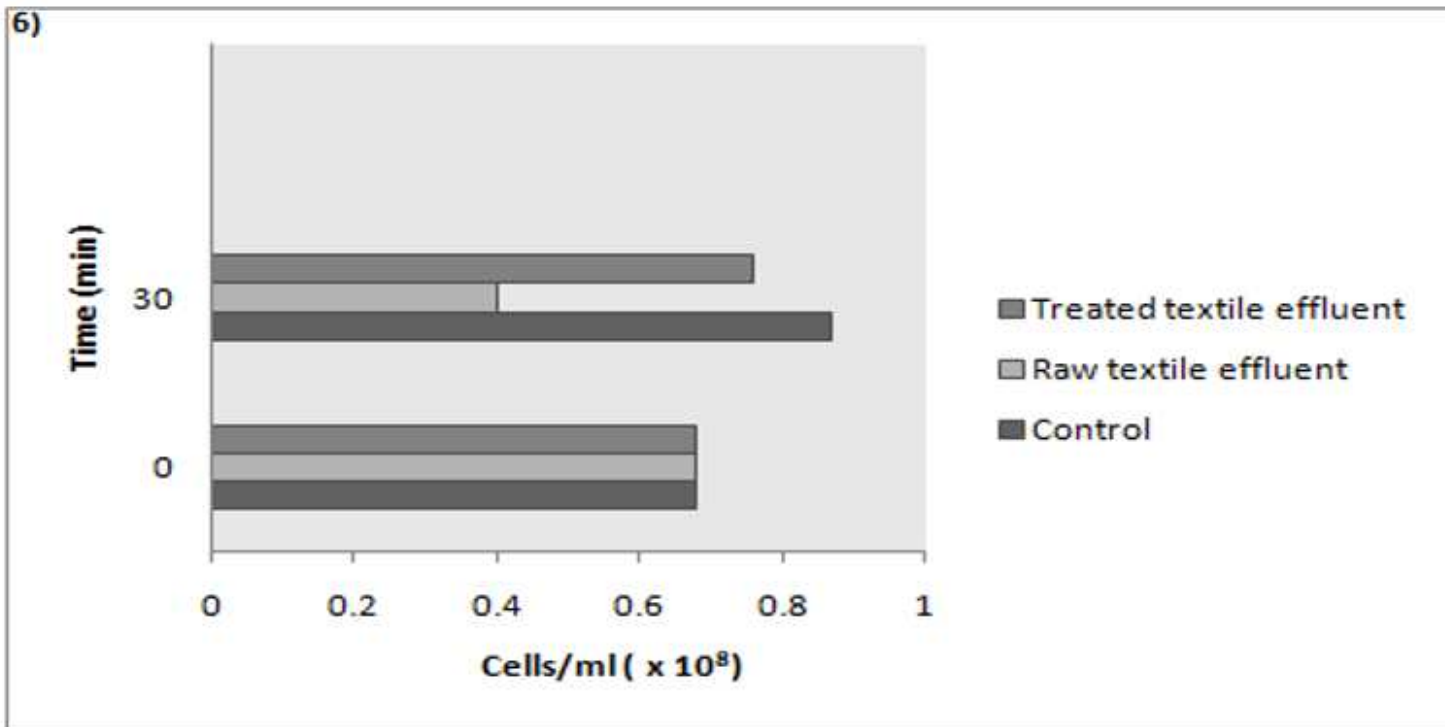
Figure 6

Microbial toxicity analysis of (a) Control (culture medium), (b) Treated textile effluent, (c) Raw textile effluent on E. coli