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Biodegradation of synthetic dye Alizarin Cyanine Green by yellow laccase producing strain *Stropharia* sp. ITCC-8422

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ABSTRACT

The dye industries have exploded tremendously since industrialization, as the consumption increases pressure upon the industries to meet the demands. Thereby leading to release of toxic chemicals and effluents in the surrounding environment. Alizarin Cyanine Green (ACG) a toxic dye has its utility in various industries and after utilization remaining dye and intermediates are released as effluent. The treatment of ACG using photocatalytic mechanism has been studied however studies via biological treatment are still lacking. Thus, the present study emphasizes on biological treatment of ACG using yellow laccase producing strain *Stropharia* sp. ITCC-8422. The strain produced 13.5 U/L of yellow laccase and efficiently decolorized ACG (200 ppm) on the second day. The dye decolorization media was optimized using OFAT to determine maximum decolorization. The role of yellow laccase on ACG decolorization was determined and maxiumum decolorization at pH 5 (87.6%) and 40 °C (73.9%) was obtained after 10 min of incubation. The control and treated ACG was analysed for dye degradation by UV-spectra, XRD and FTIR and the presence of the compounds were confirmed by HR-LCMS. The ACG (mass = 622.574) was biodegraded into low molecular weight compounds (E)-2-methylglutaconic acid (m/z = 149.0213; mass = 144.0426), ethosuximide (m/z = 146.0585; mass = 141.0798) and leucinic acid (m/z = 155.0685; mass = 132.0793). Further the phytotoxicity studies revealed that the toxicity of treated ACG reduced as compared to untreated ACG.

1. Introduction

The increase in demand of dye products has led to the production and utilization of synthetic dyes at an uncontrolled rate. The utilizations of dye require large volumes of water and is released in the environment without treatment thereby polluting it with various contaminants e.g. complex mixtures of dyes, organic compounds and salts (Adinew, 2012). The dye stuff (~20%) and reactive dye released in the environment after the dyeing process passes through the sewage treatment plants unchanged, which renders the water useless for further use and simultaneously harms the flora, fauna and water bodies (Ryan et al., 2003). The colour index (C.I.) of the dye determines its utility in different sectors and have been classified as acidic/anionic, basic/cationic, direct, disperse, mordant, reactive, solvent and vat dyes (Hunger, 2003). These dyes depending upon the structure can be further classified as i.e., azoic, anthraquinonic, heteropolyaromatic, aryl methane, xanthene, indigo etc (Vinu et al., 2010). The dye when in contact with human can cause health issues like cancer, eye irritation and chemical burn. ACG an anthraquinone dye has its utility in plastic, leather industries and as basic colouring agent in textile industries. ACG is highly toxic in nature and has detrimental effect both on human and environment. Till date photocatalytic degradation of ACG has been reported, as this method involves the used of expensive chemicals e.g. TiO_2 and photoreactors vessels for the reaction to occur, it raises the concern of the process being ecofrienldy and cost effective (Patel et al., 2016). Thus, the focus has now diverted towards the biological treatment, as these methods are highly stable and requires mild conditions to function (Couto and Herrera, 2006). Fungi are well known for the removal of various recalcitrant compounds (e.g., dyes, PAHs) due to its enzymatic machinery (Aranda, 2016). Various enzymes are known to degrade dye (e.g., laccase, manganese peroxidase etc) amongst which laccase a p-diphenol oxidoreductase belonging to the family of multi-copper protein is the most promising enzyme (López et al., 2004; Hoegger et al., 2006). The most commonly studied is blue laccase, however other type i.e., "yellow laccase" has not been studied in detail and few strains reported to produce yellow laccase are *S. aeruginosa* (Daroch et al., 2014), *T. hirsute* (Chaurasia et al., 2014), *L. squarrosulus* (Mukhopadhyay and Banerjee, 2015), *P. tigrinus* (Leontievsky et al., 1997a), *G. fornicatum* (Huang et al.,

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2011), *A. pullulans* (Ademakinwa and Agboola, 2016), *L. gongylophorus* (Ike et al., 2015), *F. durissimus* (Sahay et al., 2012), *D. flavida* (Sharma et al., 2016) and *P. ostreatus* (Pozdnyakova et al., 2004). Previously it was suggested that the yellow laccase lack blue colour due to the binding of aromatic products of lignin degradation with the blue laccase (Pozdnyakova et al., 2004). However latest research have suggested that the yellow laccase are formed due to the alterations in the catalytic copper coordinating sphere (Mate et al., 2013). They have a broader substrate specificity in comparison to the blue laccase (Leontievsky et al., 1997b), thus enabling its effective utilization in various environmental and biotechnological sectors.

In the present study an attempt was made where yellow laccase producing strain *Stropharia* sp.ITCC- 8422 isolated from extreme environmental conditions of Rajasthan was used to degrade ACG. As per best of our knowledge this is the first report on biodegradation of ACG using yellow laccase producing strain *Stropharia* sp. ITCC-8422 as till date only the photocatalytic degradation has been reported. The effect of various nutritional (carbon and nitrogen source) and physiological (pH and temperature) parameters on the dye decolorization ability was determined using One-Factor-at-a-Time (OFAT) approach, dye decolorizing efficiency of *Stropharia* sp. ITCC-8422 and role of yellow laccase on ACG decolorization was also determined. The untreated and treated ACG were further analysed via UV–visible spectra, X-ray diffraction (XRD) and Fourier-transform infrared spectroscopy (FTIR). Further the confirmation of the degradation of ACG was performed by HR-LCMS and a biodegrdative pathway was proposed and the phytotoxicity assay was carried out of degraded products.

2. Materials and methods

2.1. Materials

The chemicals used in the study were of analytical grade, 2, 2-azino-bis (3-ethylbenzothazoline-6-sulfonate) (ABTS) and ACG was purchased from Sigma and Hi-Media, India. All other chemicals were of analytical grade and purchased from, Himedia, SRL and Merck, India.

2.2. Organism and culture condition

As reported in our previous paper Agrawal et al. (2019) total fourteen (14) strains were isolated from Shreenagar, Bandarsindari Ajmer and Sanganer Jaipur, Rajasthan, India. The sample S14 collected from Bandarsindari (Latitude26°37′46.3″ N; Longitude 75°01′53.7″E) India was isolated from mushroom sample and maintained on malt extract agar (MEA) plates at 4 °C. The strains were subjected to laccase assay and were grown on ortho-dianisidine supplemented MEA plate and incubated at 28 ± 2 °C for 9 days. The presence of brown zone around the colony confirmed the presence of laccase and thus was considered for further study. The strain was further identified at Indian Agricultural Research Institute, (IARI) New Delhi India.

2.3. The primary screening for dye decolorization by *Stropharia* sp. ITCC-8422

The primary screening of the dye decolorization ability was determined using ACG, Azure B and Aniline Blue at 50 ppm in the basal salt media (BSM). The BSM consisted of the following g/L: KH_2PO_4- 7, K_2HPO_4- 2, $MgSO_4.7H_2O$- 0.1, $(NH_4)_2SO_4$- 0.1, yeast extract- 0.6, glucose- 10 at pH 5 ± 0.5 with slight modifications (Verma and Madamwar, 2002). The Erlenmeyer flask (250 mL) containing (50 mL) media was inoculated with two cubes (6 mm) and incubated at 28 ± 2 °C for 10 days. The dye decolorization was determined using UV–visible spectrophotometer (Halo- DB, Dynamica Asia Limited Hong Kong) at a wavelength range of 200–800 nm. It was then followed by determining dye decolorization percentage using utilising lambda max ($\lambda_{\max}$) of the respective dye from equation (A1):

$$Dye\ Decolorization\ (\%) = \left[\frac{Ai - Af}{Ai}\right] * 100 \qquad \text{(A1)}$$

Where Ai is the initial absorbance before dye decolorization; Af is the final absorbance after dye decolorization.

2.4. Laccase assay and UV–visible spectra of yellow laccase from *Stropharia* sp. ITCC-8422

Laccase activity determination was done quantitatively by measuring absorption change of ABTS at 420 nm, $\varepsilon_{420} = 36{,}000\ M^{-1}cm^{-1}$ (Bourbonnais et al., 1995), dissolved in sodium acetate buffer (0.1 M) pH 5 for 5 min using UV–visible spectrophotometer (Halo- DB, Dynamica Asia Limited Hong Kong). One unit of enzyme activity is defined as the amount of enzyme oxidizing 1 μmol of substrate min^{-1}. The UV–visible spectrum of the culture supernatant of *Stropharia* sp. ITCC-8422 was measured in the range of 200–800 nm (Leontievsky et al., 1997a) using UV–visible spectrophotometer (Halo- DB, Dynamica Asia Limited Hong Kong) for the presence of yellow laccase.

2.5. Optimization of nutritional and physical parameters of dye decolorization media by OFAT

The optimization of the various nutritional and physical parameters was carried out using OFAT. Total of four carbon and nitrogen sources were optimized along with pH and temperature to determine the optimum dye decolorization.

2.5.1. Effect of different carbon and nitrogen sources on dye decolorization

The four carbon sources selected for the optimization of dye decolorization media were glucose, sucrose maltose and starch at a concentration of 10 g/L and nitrogen sources were yeast, beef, malt extract and peptone at a concentration of 0.6 g/L. The Erlenmeyer flask (250 mL) containing (50 mL) media was inoculated with two cubes (6 mm) and incubated at 28 ± 2 °C for 48 h.

2.5.2. Effect of pH and temperature on dye decolorization

The effect of pH on dye decolorization was determined at a range of 3–11 and the effect of temperature was determined at a temperature range of 20–50 °C. The Erlenmeyer flask (250 mL) containing (50 mL) media was inoculated with two cubes (6 mm) and incubated at 28 ± 2 °C for 48 h.

2.6. The efficiency of *Stropharia* sp. ITCC-8422 in dye decolorization at various concentrations

The dye decolorization efficiency of *Stropharia* sp. ITCC- 8422 was determined at a concentration range of 50–600 ppm. The Erlenmeyer flask (250 mL) containing (50 mL) optimized media and dye was inoculated with two cubes (6 mm) and incubated at 28 ± 2 °C for 48 h. The dye decolorization was determined using UV–visible spectrophotometer (Halo- DB, Dynamica Asia Limited Hong Kong) at wavelength range (200–800 nm) and Eq (A1) was used to determine the percentage dye decolorization.

2.7. Role of yellow laccase from *Stropharia* sp. ITCC 8422 in decolorization of ACG

The cell free supernatant of *Stropharia* sp. ITCC 8422 was used to determine the role of yellow laccase for ACG decolorization. The effect on pH (3–11) was determined using 100 mM of sodium acetate buffer (pH 3–5), phosphate buffer (pH 7) and glycine buffer (pH 9–11). The final reaction volume (1 mL) consisting of ACG, respective buffer and yellow laccase 0.164 U/mL was incubated for 10 min. The control consisted of heat inactivated enzyme and ACG in place of active enzyme.

The absorbance was recorded at λ_{max} before and after decolorization and dye decolorization was calculated using Eq. (A1). The effect of temperature (20–50 °C) was determined by incubating ACG in enzyme under optimum pH. The absorbance of the control and treated ACG along with the calculation of dye decolorization was done as mentioned previously.

2.8. Analysis of dye and dye degraded products by UV–visible spectra, XRD, FTIR and HR-LCMS

The UV–visible spectra of the dye degraded products were determined at a wavelength of 200–800 nm using UV–visible spectrophotometer (Halo- DB, Dynamica Asia Limited Hong Kong). The dye degraded product was first extracted with equal volumes of ethyl acetate and dried to powder form in rotary evaporator for XRD, FTIR and HR-LCMS. The XRD analysis was done using PANalytical EMPYREAN with Cu anode material. The dried samples were scanned within 2θ range of 10–80°. The FTIR analysis was done at IIT Bombay, Sophisticated Analytical Instrument Facility (SAIF) and the analysis was performed at a wavenumber range of 400–4000 cm^{-1}. The HR-LCMS for the analysis of the dye degraded products was performed using column: zorbax SB c18, 2.1 × 150 mm 1.8-micronat, at IIT Bombay, SAIF.

2.9. Phytotoxicity assay of the dye degraded products

The toxicity of untreated and treated ACG was determined by phytotoxicity assay using *Vigna radiata*. The dye and dye degraded product were centrifuged at 12,000 rpm and 1 mL was added to the soil and the control consisted of tap water. Ten (10) seeds were sown in the pot and the assay was performed for 10 days at 30–35 °C with the addition of 5 mL of water every 24 h (Chandra et al., 2008; Azmat et al., 2006). All the experiments were carried out in triplicate. On the 10th day the length of the root and the shoot were measured and the germination index was calculated using equation (B1):

$$Germination\ Index = (G^{*}L)/(Gc^{*}Lc) \tag{B1}$$

Where G and L germination and radical growth of the seed in treated ACG solution and Gc and Lc is germination and radical growth of the seed in control ACG.

3. Result and discussion

3.1. Isolation, identification and screening of laccase positive strain

The isolated strains were subjected to primary laccase assay and the strain designated as S14 exhibited positive activity with ortho-dianisidine as the substrate and was considered for further study (Fig. S1). The strain S14 was then identified as *Stropharia* sp. by Indian Type Culture Collection (ITCC), Indian Agricultural Research Institute (IARI), New Delhi and later the culture was deposited at ITCC-IARI with Accession number: ITCC-8422.

3.2. The primary screening for dye decolorization by Stropharia sp. ITCC-8422

The primary dye decolorization assay was performed using three dyes and maximum decolorization i. e 100% was obtained with ACG followed by Azure B 25.6% after 48 h of incubation. However, no decolorization was observed with Aniline Blue (Table 1). Thus, further study was carried out using ACG (Fig. S2 a-c).

3.3. Laccase assay and UV–visible spectra of yellow laccase from Stropharia sp. ITCC-8422

The maximum laccase production was achieved on 18th day i.e., 164.4 U/L, however the enzyme production on first day was 1.6 U/L and on the second day its increased to 13.5 U/L which further increased up to 18th day and then gradually declined (Fig. 1a).

Table 1
Primary screening for the dye decolorization ability of yellow laccase producing strain *Stropharia* sp. ITCC-8422.

Sl. No.	Dyes	Groups	C.I No.	Decolorization (%)
1	Alizarin Cyanine Green	Anthraquinone	61570	100
2	Azure B	Cationic (Basic) dye	52010	25.6
3	Aniline Blue	Acidic (Anionic) dye	42780	ND

ND-no decolorization.

The UV–visible spectrum of laccase did not have the presence of absorption maximum at 610 nm which is responsible for the “blue maximum” in case of blue laccase. Thus, confirming the presence of yellow laccase in *Stropharia* sp. ITCC-8422 (Fig. 1b). Similar results was observed in case of *P. tigrinus* (Leontievsky et al., 1997a) and in the work done by Daroch et al. (2014) the basidiomycete strain *Stropharia aeruginosa* was reported to produce glycosylated yellow laccases.

3.4. Effect of different carbon and nitrogen sources on dye decolorization by Stropharia sp. ITCC-8422

It was an attempt for use of different carbon sources to obtain maximum dye decolorization efficiency of *Stropharia* sp. ITCC-8422 at 200 ppm. It was observed that on the second day maximum decolorization was obtained with sucrose 97.4% followed by 97.1% for both glucose and maltose and finally by starch 74.2% (Fig. 2a). It was observed that the most efficient carbon source was sucrose, similar studies are also reported where highest Malachite Green decolorization was observed in the presence of sucrose (Barapatre et al., 2017).

Similarly, various nitrogen sources were also optimized and it was observed that on the second day maximum decolorization was obtained with malt 98.2% followed by beef and yeast extract 97.6% and 97.1% and finally by peptone 96.3% (Fig. 2b). It was observed that the most efficient nitrogen source was malt, followed by beef and yeast extract the least favorable was peptone.

3.5. Effect of pH and temperature on dye decolorization of ACG by Stropharia sp. ITCC-8422

It has been reported that fungi exhibits better dye decolorization ability at acidic or neutral pH and the pH of the media influences the decolorizing efficiency of fungal cultures (Khan et al., 2013; Park et al., 2007). It was observed that *Stropharia* sp. ITCC-8422 exhibited maximum decolorization at pH 5 followed by pH 3 and pH 7 exhibiting decolorization of 97.1%, 39.3% and 22.1% respectively. As the pH increased from 9 to 11, percentage decolorization decreased to 10.3% and 6.1% respectively (Fig. 2c).

The effect of temperature on dye decolorizing ability was studied and it was observed that most efficient decolorization i.e., 100% was at 40 °C followed by 97.1% and 91.9% at 30 °C and 20 °C respectively. It was observed that at 50 °C the dye decolorizing efficiency of the strain reduced to 14.1% suggesting the decrease in efficacy of the strain at higher temperature (Fig. 2d). More than 90% dye decolorization was obtained at a temperature range of 20–40 °C after 48 h of incubation.

3.6. The efficiency of Stropharia sp. ITCC-8422 in dye decolorization at various concentrations

In order to determine the dye decolorizing ability of the yellow laccase producing strain *Stropharia* sp. ITCC-8422, it was inoculated at

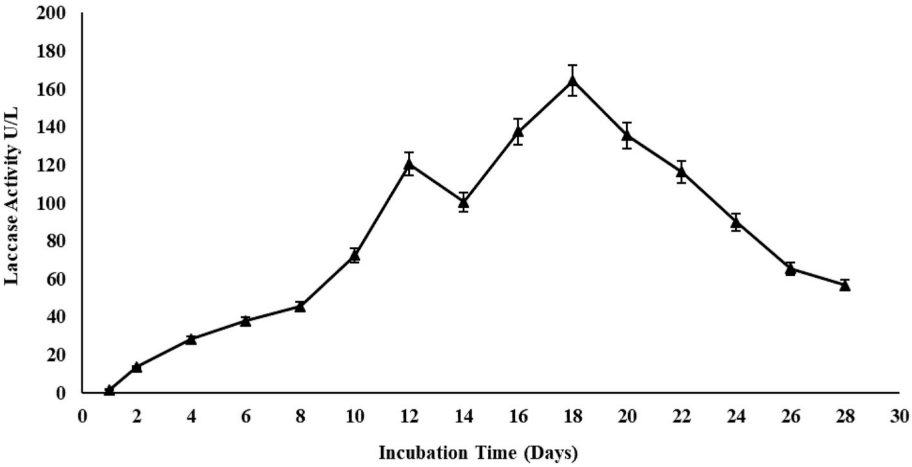

Fig. 1a. Enzymatic profiling of laccase activity using *Stropharia* sp. ITCC-8422 incubated at 28 °C, pH 5 for 28 days.

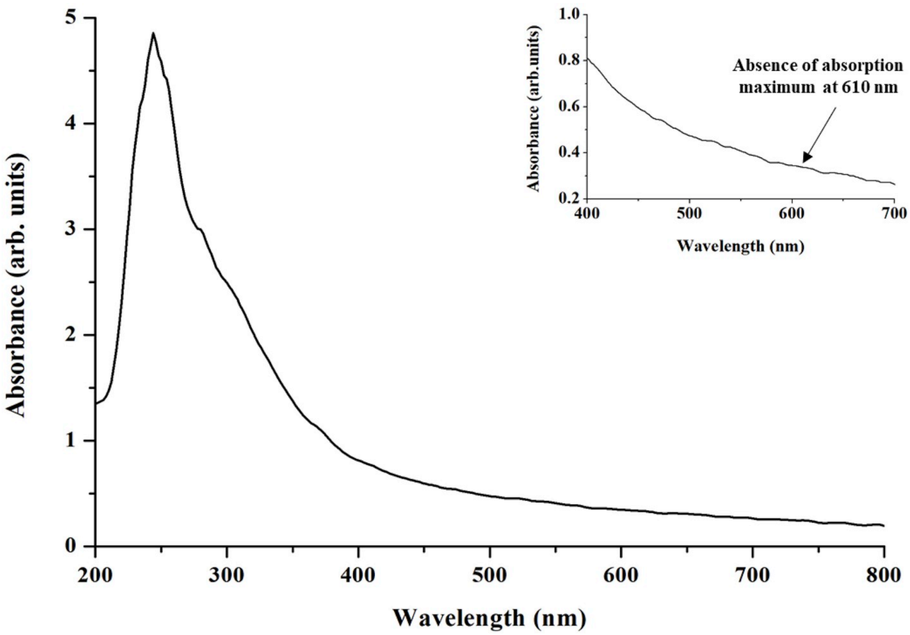

Fig. 1b. The UV–visible spectra of yellow laccase from the culture supernatant of *Stropharia* sp. ITCC-8422.

an increasing concentration of ACG i.e., 50–600 ppm. It was observed that 100% decolorization was attained after 48 h of incubation at 50–250 ppm. However, > 90% decolorization was attained by *Stropharia* sp. ITCC-8422 at a (300–600 ppm) concentration range (Fig. 3). Thus, the strain efficiently decolorized ACG (600 ppm) within 48 h of incubation.

3.7. Role of yellow laccase from Stropharia sp. ITCC 8422 in decolorization of ACG

The maximum decolorization was obtained at pH 5 (87.6%) followed by pH 7 (72.3%) and pH 9 (63%). The efficiency of decolorization decreased at pH 3 (19.5%), whereas no decolorization was obtained at pH 11 (Fig. 4a). Thus, it was observed that pH affected the dye decolorizing efficiency of yellow laccase (Nyanhongo et al., 2002). On the other hand incase of temperature maximum decolorization was obtained at 40 °C (73.9%) and above 60% decolorization was obtained at 20 °C, 30 °C and 50 °C (Fig. 4b). It was observed that in both the cases of control consisting of heat inactivated enzyme and ACG no decolorization was observed.

3.8. UV–visible spectra of dye degraded products

The initial dye degradation study was carried out using UV–visible spectrophotometer. It was observed that at λ_{max} 644 nm complete dye degradation was observed after 48 h of incubation as the peak disappeared completely. As per Chen et al. (2012) the band at 644 nm represents the chromophore part in the dye molecule, the total decrease in the peak represents the destruction of the chromophore part of the dye. In Fig. 5 a peak at 248 nm increased during the incubation period and after 48 h a shift in peak was observed from 248 to 244 nm. Similarly, at 298 nm at 24 h the peak height increased and at 48 h the peak height decreased and the shift in peak was observed at both 24 and 48 h from 298 to 294 nm. The other shift in peak was from 416 to 406 nm where the peak decreased and shifted only after 48 h of incubation. The decrease in peak height at the UV region may be due to the opening reaction of the aromatic rings (Chen et al., 2012). As per Ayed et al. (2009) it has been stated that the shift of the peak in dye degradation studies are responsible for the formation of new compounds and if the decrease in peak height is proportional to the time of incubation the mode of action on the dye is decolorization, however if the peaks

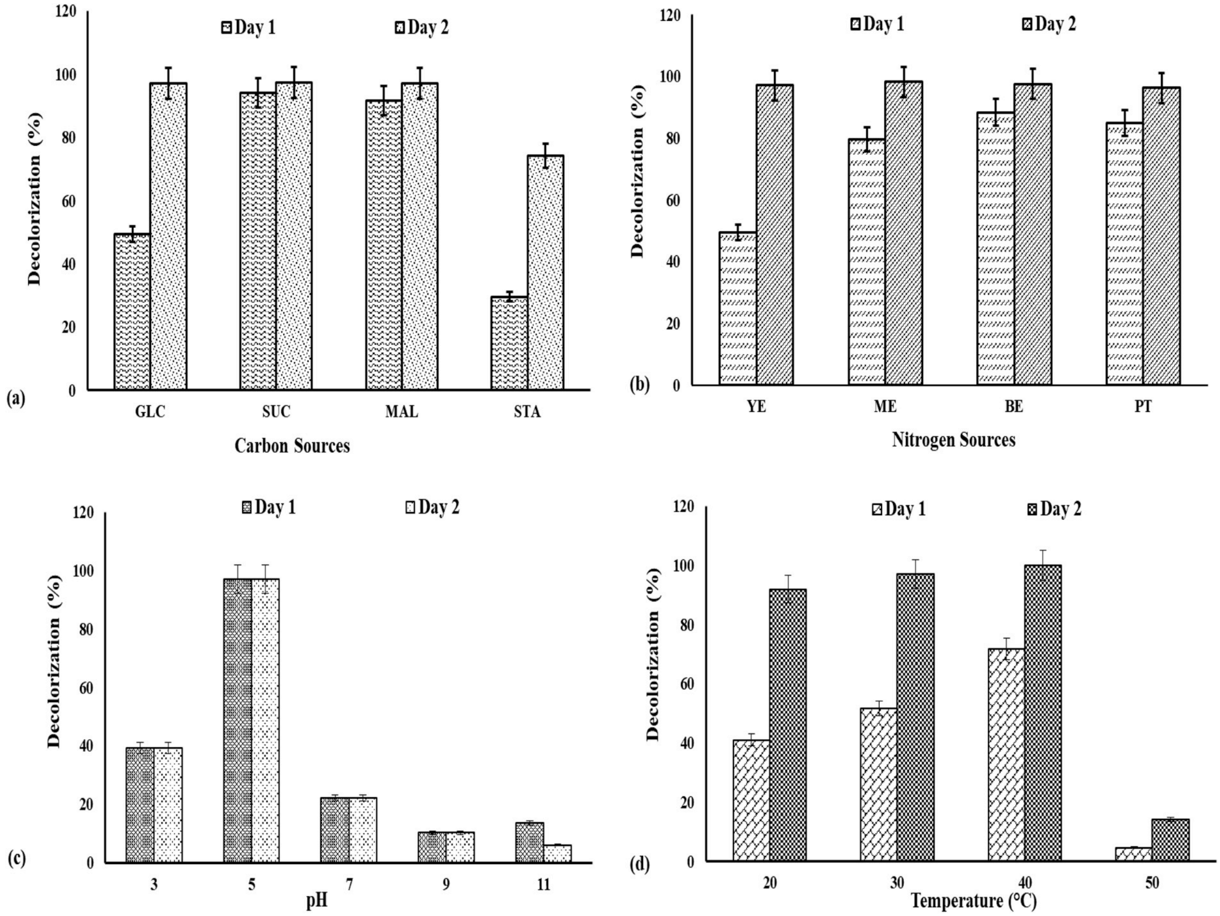

Fig. 2. The optimization of various nutritional and physical parameters using One-Factor-at-a-Time (OFAT) for the efficient dye decolorization of Alizarin Cyanine Green (ACG) using *Stropharia* sp. ITCC-8422 (a) Carbon; (b) Nitrogen; (c) pH and (d) Temperature.

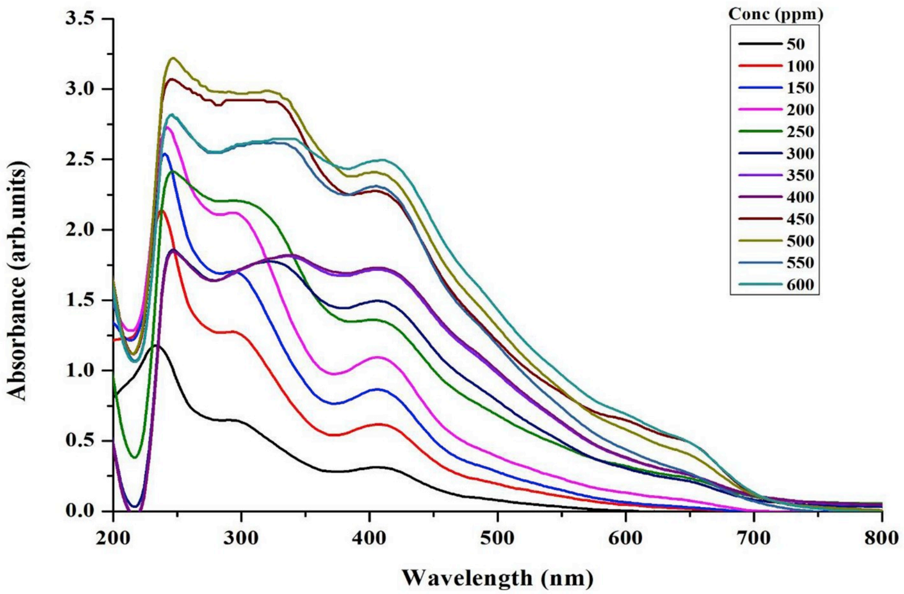

Fig. 3. UV–visible spectra of dye degradation efficiency of *Stropharia* sp. ITCC-8422 at various concentrations (50–600 ppm) of Alizarin Cyanine Green (ACG) under optimized condition after 48 h of incubation.

disappear completely or new peaks appear dye degradation has occurred instead of decolorization. As in the present study peak has completely disappeared at 644 nm and new peaks have appeared at 244, 294 and 406 nm, degradation is the mode of action on the dye by yellow laccase producing strain *Stropharia* sp. ITCC- 8422.

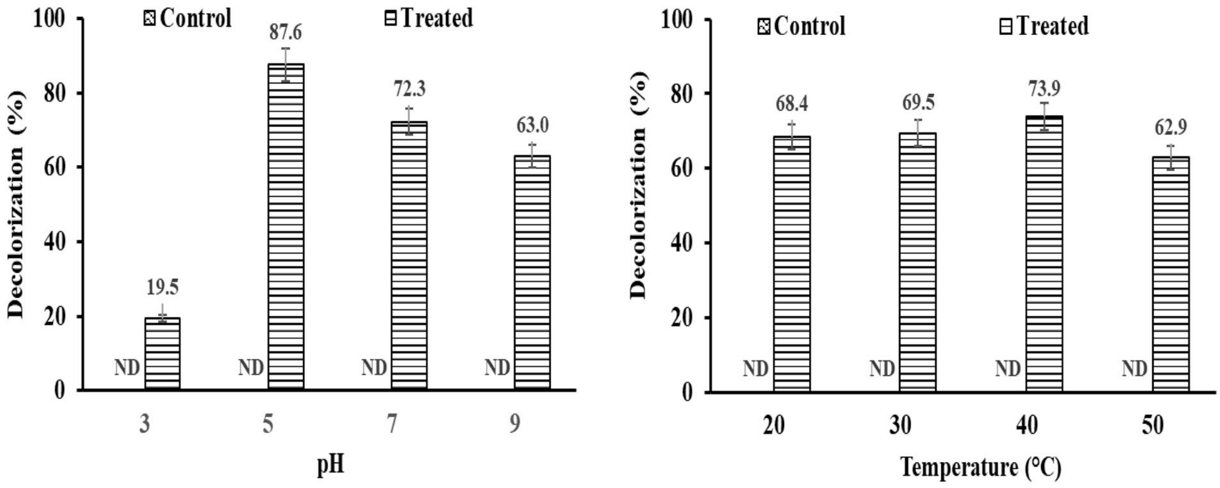

Fig. 4. The role of yellow laccase on decolorization of ACG at various (a) pH (3–9) and (b) Temperature (20–50 °C) after 10 min of incubation. The control consisted of heat inactivated enzyme and ACG (ND-no decolorization).

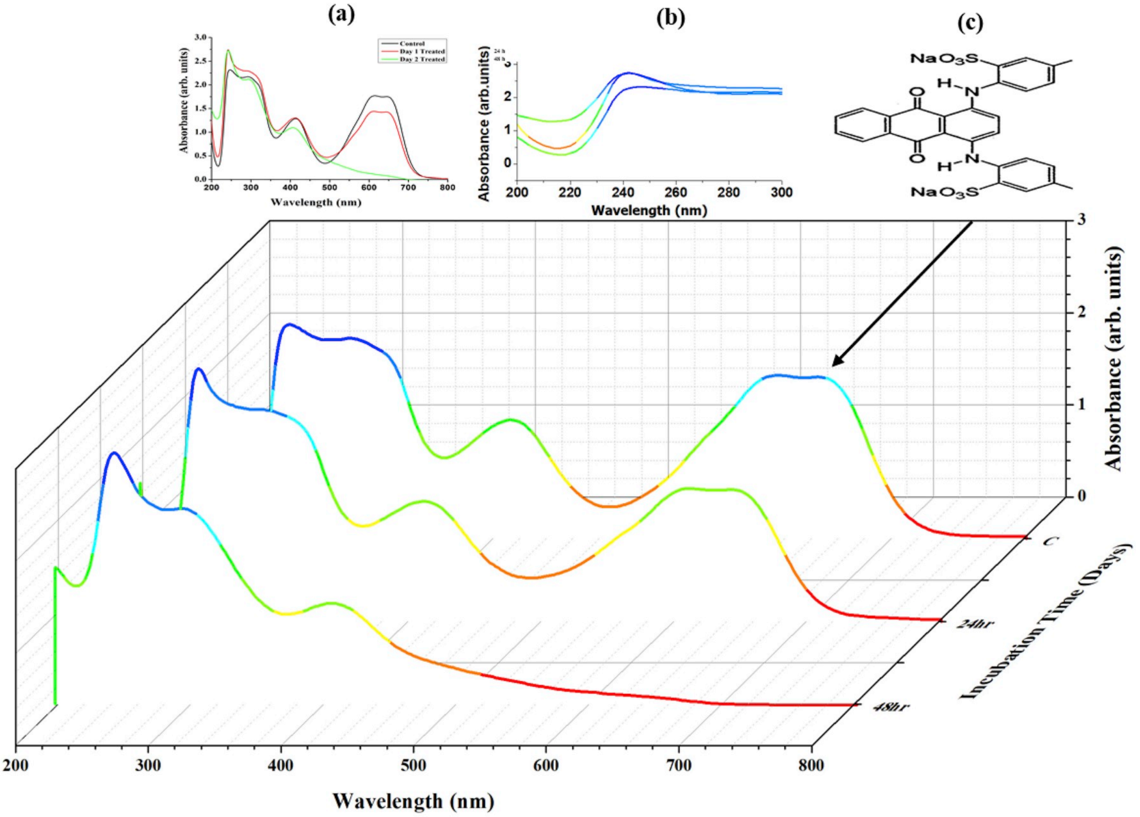

Fig. 5. UV–visible (3D) spectra of Alizarin Cyanine Green (ACG) both control and treated by *Stropharia* sp. ITCC-8422 after 24 and 48 h of incubation, insert image (a) UV-spectra; (b) peaks of control and treated ACG in the range of 200–300 nm; (c) maximum absorbance (λ_{max}) and structure of ACG.

3.9. XRD of dye degraded products

The XRD pattern helps to analyze the crystalline and amorphous nature of the material, where the crystalline systems give well-defined peaks the amorphous systems give hallow peaks (Cullity, 1987). The XRD data has revealed that the most of the crystalline peaks having strong intensity in case of control ACG disappeared completely or the intensity decreased after the treatment with yellow laccase producing strain *Stropharia* sp. ITCC- 8422. At degree 2θ (10.46, 12.97, 13.26, 13.70, 15.74, 15.79, 19.11, 23.29, 23.51, 24.13, 24.54, 24.69, 25.44, 27.45, 28.11, 29.09, 31.76, 32.18, 45.51) the intensity decreased tremendously after the treatment or the peaks disappeared completely (Fig. 6).

3.10. FTIR of dye degraded product

The FTIR spectra showed the peak in the range of 3500–3304 cm^{-1} (-NH stretch) increased in treated ACG and at 1606-1480 cm^{-1} (aromatic) peak was visible in control ACG however in treated ACG the peak decreased. Similarly, the peak at 745-895 cm^{-1} represents aromatic vibrations which was more in case of the treated ACG in comparison to the control ACG (Kolekar et al., 2013). As per Seferoğlu et al. (2008) the

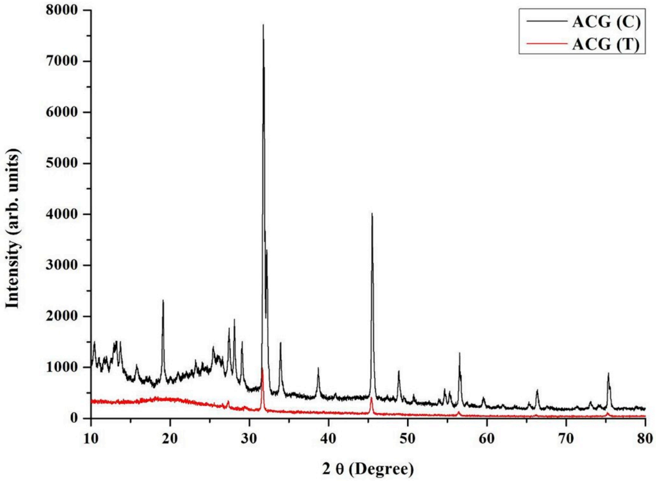

Fig. 6. XRD of Alizarin Cyanine Green (ACG) both control and treated by *Stropharia* sp. ITCC-8422 under optimized condition after 48 h of incubation.

bands at 3462-3180 cm^{-1} represents –NH band with indole ring, 3418-3264 cm^{-1} represents –SH and –NH bands of the triazole ring, 2986-2851 cm^{-1} represents aliphatic CH, and 3085-3005 cm^{-1} represnts aromatic CH, these bands were more in intensity in the treated ACG than the control ACG. According to Yani and Zhang (2010) the bands at 610–680 cm^{-1} represents ion sulphate which is prominently present in control ACG and has been effectively been removed in the treated ACG. It was observed that the peaks at 1140 cm^{-1} (triply degenerate symmetric stretching mode of SO4 $^{2-}$), and 631.6 cm^{-1} (triply degenerate vibrations of SO4 $^{2-}$) was effectively removed in case of the treated ACG as compared to the control however the peaks has 461 cm^{-1} (the doubly degenerate SO4 $^{2-}$), and 984.6 cm^{-1} (SO4 $^{2-}$ non degenerate mode) had no effect either in the control or treated ACG (Fig. 7) (Yani and Zhang, 2010). Thus, as per the above analysed data and as per Kolekar et al. (2013) these changes confirm the effective biodegradation of ACG in the presence of *Stropharia* sp. ITCC-8422**.**

3.11. HR-LCMS of dye degraded products

In order to identify the degraded compounds HR-LCMS was performed. It was observed that the initial molecular weight of ACG was 622.574 g/mol, and few degraded products have low molecular weight in comparison to the control ACG (Table S1) and based on the above analysis a possible biodegradative pathway of ACG has been predicted (Fig. 8). As per Meng et al. (2003) the anthraquinone dye which are green violet and blue have long term colour stability. Laccase is capable to degrade anthraquinone dye more effectively than other classes of dye and during the biodegradation by laccase the anthraquinone molecules form low molecular weight structure with low toxicity (Zeng et al., 2011, 2012). The biodegradation of ACG by *Stropharia* sp.ITCC-8422 consisted to three predictive pathways where ACG (mass = 622.574) were oxidized and reduced to low molecular weight compounds baeomycesic acid (m/z = 379.0735; mass = 374.0948), 3-Hydroxy-MEGX (m/z = 245.127; mass = 222.1376) in the first, desmethylindomethacin (m/z = 348.0374; mass = 343.0585), nefopam (m/z = 236.1464; mass = 253.1497) in the second and hydroxydiphenoxylic acid (HDPA) (m/z = 445.1874; mass = 440.2077) and amobarbital (m/z = 227.1367, 227.1373; mass = 226.1294, 226.1299) in the third. These compounds (Fig. S3) from each pathway were further converted to (E)-2-methylglutaconic acid (m/z = 149.0213; mass = 144.0426), ethosuximide (m/z = 146.0585; mass = 141.0798) and leucinic acid (m/z = 155.0685; mass = 132.0793). The data of the UV–visible spectra, FTIR, XRD and HR-LCMS further concludes that the dye has been effectively biodegraded in the presence of yellow laccase producing strain *Stropharia* sp. ITCC-8422.

3.12. Phytotoxicity assay of the dye degraded products

The dye stuff which is released in the environment causes harsh effect on the flora. In general, the present study showed no significant effect on the germination index of untreated and treated ACG. However, at certain concentrations it was observed that the fungal treated dye had positive impact (Table 2) on the growth of root and shoot length (Kalyani et al., 2008). It may be due to the presence of useful chemical intermediates in the treated ACG as compared to untreated ACG.

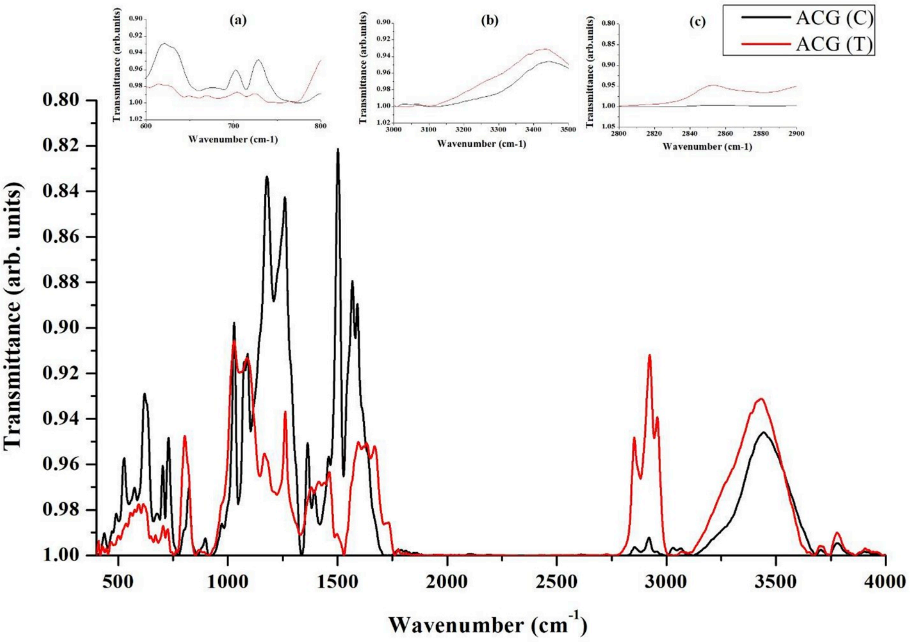

Fig. 7. FTIR spectra of Alizarin Cyanine Green (ACG) both control and treated by *Stropharia* sp. ITCC-8422 under optimized condition after 48 h of incubation, insert image of control and treated ACG of various wavenumber ranges (a) 600-800 cm^{-1}; (b) 3000-3500 cm^{-1} and; (c) 2800-2900 cm^{-1}.

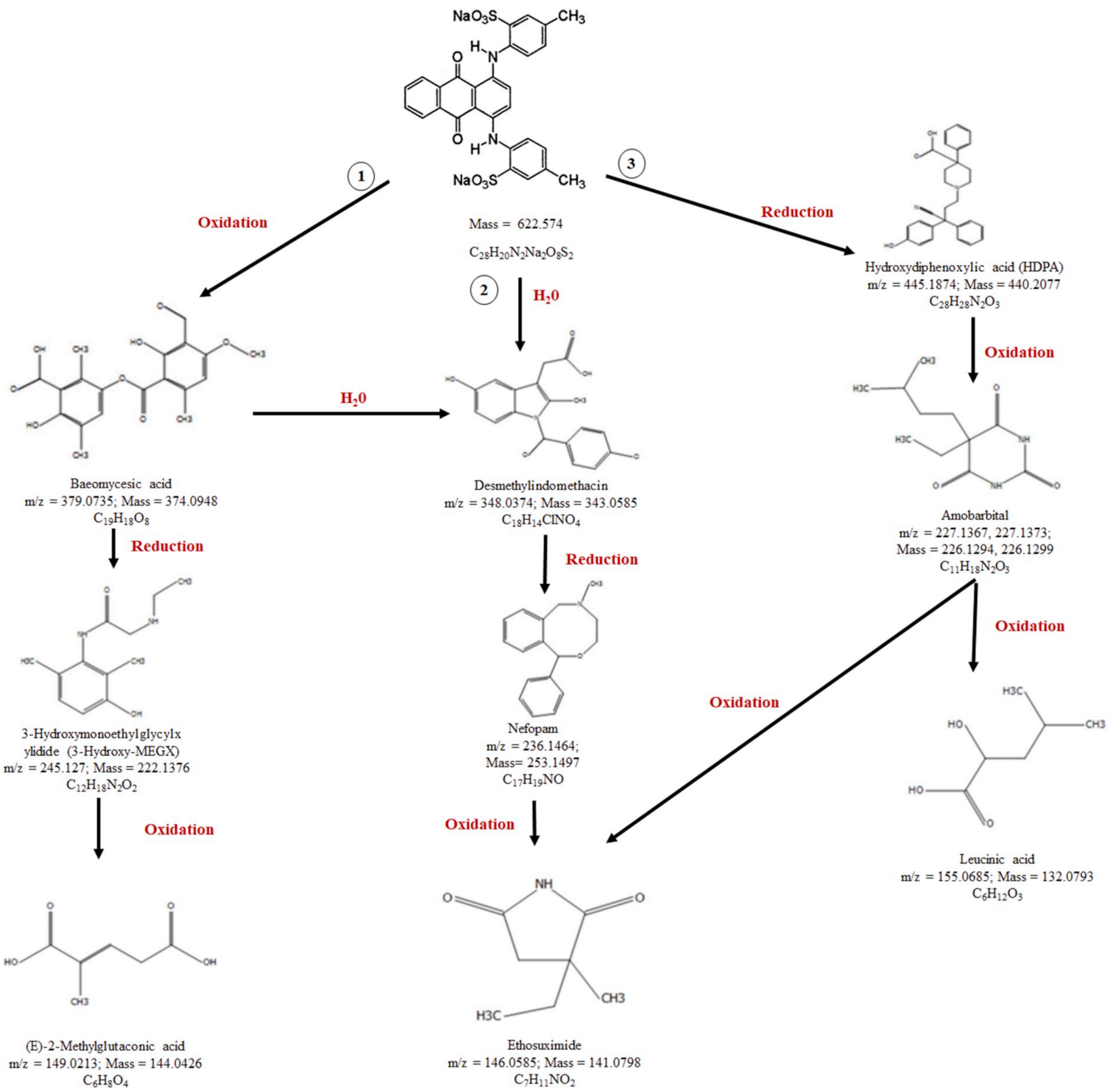

Fig. 8. The proposed pathway for the biodegradation of Alizarin Cyanine Green (ACG) by yellow laccase producing strain *Stropharia* sp. ITCC-8422 on the basis of various degraded products of low molecular weight detected by High-Resolution Liquid Chromatograph Mass Spectrometer (HR-LCMS).

Table 2
The phytotoxicity of the Alizarin Cyanine Green (ACG) and its degraded products treated by yellow laccase producing strain *Stropharia* sp. ITCC-8422 under controlled conditions.

Dye range (ppm)	Untreated		Treated		Untreated dye (GI)	Treated dye (GI)
	Root Length(cm)	Shoot length(cm)	Root Length(cm)	Shoot length(cm)		
50	9.3 ± 0.5	20.7 ± 0.5	8.3 ± 0.5	19.0 ± 0.3	3.0 ± 0.5	2.4 ± 0.4
100	1.2 ± 0.6	20.3 ± 0.4	5.5 ± 0.4	15.7 ± 0.3	0.4 ± 0.4	1.3 ± 0.3
150	8.7 ± 0.4	18.3 ± 0.6	8.0 ± 0.6	20.3 ± 0.4	2.4 ± 0.4	2.5 ± 0.4
200	3.7 ± 0.5	18.8 ± 0.5	7.3 ± 0.4	17.7 ± 0.6	1.1 ± 0.5	2.0 ± 0.5
250	9.7 ± 0.5	17.5 ± 0.4	4.3 ± 0.5	12.3 ± 0.4	2.6 ± 0.4	0.8 ± 0.4
300	4.7 ± 0.6	21.0 ± 0.6	5.3 ± 0.4	17.7 ± 0.4	1.5 ± 0.6	1.4 ± 0.4
350	5.3 ± 0.5	10.7 ± 0.4	3.8 ± 0.6	15.3 ± 0.5	0.9 ± 0.4	0.9 ± 0.5
400	7.8 ± 0.3	17.7 ± 0.5	5.5 ± 0.4	19.7 ± 0.5	2.1 ± 0.4	1.7 ± 0.4
450	8.0 ± 0.6	19.5 ± 0.6	9.3 ± 0.4	15.0 ± 0.3	2.4 ± 0.6	2.2 ± 0.3
500	8.0 ± 0.3	16.9 ± 0.3	4.8 ± 0.3	21.5 ± 0.5	2.1 ± 0.3	1.6 ± 0.4
550	10.3 ± 0.6	22.7 ± 0.3	10.0 ± 0.5	23.7 ± 0.4	3.6 ± 0.4	3.6 ± 0.4
600	5.7 ± 0.3	18.9 ± 0.4	7.7 ± 0.3	21.3 ± 0.3	1.7 ± 0.3	2.5 ± 0.3

Control: Root (cm) - 5 ± 0.3: Shoot (cm) – 13 ± 0.6; GI: Germination Index.

4. Conclusion

The yellow laccase producing strain *Stropharia* sp. ITCC-8422 degraded ACG efficiently and in addition the dye and its degraded products were analysed by UV-spectra, FTIR, XRD and HR-LCMS. It was analysed that the untreated ACG was effectively biodegraded into low molecular weight products by yellow laccase producing strain *Stropharia* sp. ITCC-8422 and had positive impact on the growth of the plant *Vigna radiata* in comparison to the control ACG thereby favouring towards both "*Greener and Cleaner Environment*".

Conflicts of interest

The authors declare that they have no conflict of interest to disclose.

Declaration of interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bcab.2019.101291.

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