Bioremediation Potential of Duckweed (Lemna Gibba) in The Decolorization of C.I. Basic Green 4 and Chlorophyll a Fluorescence Analysis: Understanding Plant Performance

Hanwant Singh
Mohanlal Sukhadia University
Shani Raj
Mohanlal Sukhadia University
Deepak Kumar
Mohanlal Sukhadia University
Shubhangani Sharma
Mohanlal Sukhadia University
Upma Bhatt
Mohanlal Sukhadia University
Vineet Soni (vineetpbbl154@gmail.com)
Mohanlal Sukhadia University

Research Article

Keywords: Lemna gibba, Basic Green 4, Biodecolorization, OJIP, Phytoremediation

Posted Date: January 15th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-144187/v1

Bioremediation potential of duckweed (*Lemna gibba*) in the decolorization of C.I. Basic Green 4 and chlorophyll *a* fluorescence analysis: understanding plant performance

Hanwant Singh, Shani Raj, Deepak Kumar, Shubhangani Sharma, Upma Bhatt and Vineet Soni*

Plant Bioenergetics and Biotechnology Laboratory, Department of Botany Mohanlal Sukhadia University, Udaipur, 313001, Rajasthan, India

*Corresponding Author

Vineet Soni
Associate Professor
Plant Bioenergetics and Biotechnology Laboratory
Department of Botany
Mohanlal Sukhadia University,
Udaipur, 313001, Rajasthan, India
Email: vineetpbbl154@gmail.com

Abstract

In this research, the duckweed (*Lemna gibba*) potential has been investigated spectrophotometrically as an obvious bioagent for the biological decolorization of organic dye Basic Green 4 (BG4). Experiment result showed that *L. gibba* has a potent ability to extract BG4 from contaminated water. The study showed that for better results the temperature at 25-30 ^{o}C and pH 8.0 is considered to be optimum. A significant induction in SOD, GPOD and CAT activity was observed in *L. gibba* treated with 15 and 30 mg / l BG4, respectively, after 24 hours of biodecolorization process. It was observed during five repeated batch run that *L. gibba* showed comparable efficiency in dye decolorization. The chlorophyll fluorescence analysis also shows that photosynthetic apparatus of *L. gibba* is highly tolerant of BG4. The overall results of the observations here demonstrate the duckweed *L. gibba* can be used as a potent biodegrading organism for BG4.

Keywords: *Lemna gibba*; Basic Green 4; Biodecolorization, OJIP; Phytoremediation;

1. Introduction

With the increasing human civilization and industrialization, a lot of contaminants are discharged into the aquatic ecosystem, thereby integrating them into the food chain, which ultimately leads to deleterious effects on daily lives. Dyes have been a significant water contaminant in recent years, used for colour processing and other industrial purposes [1]. Two types of dyes are: natural and synthetic. Natural dyes are having some advantage to easily precipitate naturally. In the present day, synthetic dyes are dominant in textile industries because of their long durability, unaffected to environmental factors, and exhibit a wide range of colors [2]. A huge increase in industries and the human need for colour leads to increased everyday use of colours [3]. It was reported the concentration of dye in textile industries effluent is 300 mg/l and normally the color noticeable at a dye concentration of 1 mg/l [4] and 80,000 tons of commercially available dyestuff discharged in the wastewater drainage and contaminate the water [5]. In aquatic plants, photosynthesis is also affected by dyes contamination because it reduces the penetration of light in water [6,7]. hence, the removal of all pollutants from wastewater is the main objective for mankind [8]. The conventional wastewater treatment technologies such as physical and chemical, are more expansive and introduce other toxic byproducts which are hazardous and required further processing [9,10]. In order to overcome this problem several studies have been focused to use of bioagents such as bacteria, fungi, yeasts, and algae to remove or degrade dyes from contaminated water [10–15]. Some plant species are also reported which have dye decolorization potential [16–19].

BG4 is a cationic dye commonly used for dying silk, leather, wool, and also used as a fungicide [20]. There are many deleterious effects of BG4 were reported to animals like impair protein synthesis, muscle glycogenolysis, severe damage in fishes. BG4 also cause tumor in human beings and harmful for symbiotic bacteria found in water [21]. Leucomalachite a reduced form of

63 BG4 keeps it up in edible fish for a long time, hence there is more essential to remediate it from
64 wastewater for both environmental and human health [22,23].

65 In the present study, *L. gibba* L. was used to examine its biodecolorization potential of
66 organic dye BG4 commonly known as Malachite Green. *L. gibba* is an aquatic macrophyte belongs
67 to the family Lemnaceae and their small size, high multiplication rate, and reduced anatomy makes
68 it a good candidate for dye decolorization [24–26]. To assess the ability of *L. gibba* to decolorization
69 of BG4 various operational parameters were determined such as initial biomass concentration,
70 temperature, and pH. Additionally, we also examine the change in antioxidant activity by analyses
71 three Reactive Oxygen Species (ROS) scavenging enzymes superoxide dismutase (SOD);(EC
72 1.15.1.1.), Catalase (CAT);(EC 1.11.1.6.), and guaiacol peroxidase (GPOD);(EC 1.11.1.7). The
73 repeated batch operation was also performed to determine the reusability of *L. gibba* for dye
74 decolorization. Through the analysis of polyphasic chlorophyll fluorescence, efforts were also
75 made to study the impact of the dye decolorization process on PSII photochemistry of *L. gibba*.

76 ## 2. Materials and methods

77 ### 2.1 Plant materials and growth condition

78 In the present investigation duckweed, *L. gibba* was used for phytoremediation of
79 triphenylmethane dye BG4. Plant material **(Figure 1a)** was collected from the region of Ayad river
80 located at Udaipur, India (24°35'14.97"N, 73°42'38.75"E). Details about water properties and
81 environmental condition of Ayad river are given in **Table 1**. BG4 was procured from HiMedia
82 Pvt. Ltd. New Delhi (India). The plants were rinsed with double distilled water to remove surface
83 contamination and maintained in plastic pond under illumination provided by white fluorescent
84 light with 6500–10000 lux light irradiance, 14-h photoperiod, and 25/20 °C day/night temperature
85 for three months as a pre-treatment before experiments [27]. A full-strength Jacob culture medium

was prepared (Detail composition of media was given in **Table 2**), and the pH was adjusted to 6.0 with 0.1 M KOH, circulation provided with a pump and the medium was replaced every 2 months.

2.2 Experiment

The decolorization experiments were performed in the 250 ml beaker containing 200 ml malachite green dye **(Figure 1b).** Details about chemical structure and characterization of BG4 are given in **Table 3**. After every regular treatment interval, the sample was isolated and the remaining dye was determined with a UV spectrophotometer (Analytikjena® Specord 200, Germany) at maximum absorbance wavelength (λ_{max}) =619 nm. A linear calibration curve was plotted between the concentration of dye and the absorbance (A) at 619 nm (λ_{max}). in the range of C_{dye} = 0 to 30 mg/l. % dye removal was calculated by using Eq. (i).

$$\% \, Dye \, removal = [1 - \left(\frac{A}{Ao}\right)] \cdot 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots \text{(i)}$$

The batch decolorization experiment was carried out at different duckweed concentrations (2,4,6,8 and 10 g), temperature (10, 20, 30,40 and 50 ^{o}C), and pH value (2,3,4,5,6,7,8 and 9). The pH of the dye solution was adjusted using 1N NaOH and 1 N HCl and was measured by pH meter (Hanna HI98100, United States). To determine the reusability of *L. gibba* repeated-batch processes were performed to remove 6 g BG4 [12].

Fourier Transform Infrared (FT-IR) spectroscopy was performed according Khataee et al. (2010) by using Bruker Tensor 27 spectrometer, Germany[20]. For FT-IR analysis, biological treatment process was performed with 250 ml solution containing 10 mg/l of MG and 4 g of duckweed fronds. At the reaction times of 0 h (control), 6 h and 12 h samples were taken and the biological degradation products were extracted with 30 ml of diethyl ether in three times, then crystallized and used for analysis.

2.3 Enzyme extraction and assay

About 300 mg *Lemna* fronds (fresh weight) were homogenized in 5 ml ice-cold potassium phosphate buffer (0.1 M, pH 7.8) for prepare enzyme extract, the homogenate was centrifuged at 15,000 × g (4 ^{O}C) for 20 min (Remi®, India). the supernatant was saved and used as the enzyme extract. All the preparation for enzyme extract was carried out at 4 ^{o}C.

The SOD activity was determined by spectrophotometrically by measuring its ability to inhibit the photochemical reduction of Nitro Blue Tetrazolium (NBT) at 560 nm [28]. The reaction mixture containing 100 µl, L- methionine, 100 µl NBT, 10 µl riboflavin, and 100 µl enzyme extract. Make up the volume 3 ml by adding 0.05M Na_2CO_3.

The CAT activity was measured by the consumption of H_2O_2 at 240 nm [29]. The reaction mixture containing 120 µl enzyme extract, 80 µl H_2O_2 (500mM) and make final volume 3 ml by adding 2.8 ml potassium phosphate buffer (50mM).

GPOD activity was determined by spectrophotometrically by measuring changes in absorbance at 436 nm for 15 sec. up to 5 minutes [30]. Reaction mixture containing 1 ml guaiacol (1%), 1.7 ml phosphate buffer (0.05M, pH 7.0). the reaction started by adding H_2O_2.

2.4 Chlorophyll fluorescence kinetics

2.4.1 Fast Chl a fluorescence kinetic transient

To determine changes in Chlorophyll (Chl) *a* fluorescence O-J-I-P transient was recorded after 12 h of dye treatment by Plant Efficiency Analyzer, PEA (*Hansatech Instruments*, Kings Lynn, Norfolk, U.K.). Before the measurements, control and treated plants were adapted to darkness in room for 1 h, and additionally, the measured spots were kept in darkness in the clip for 1 min just before measurement [31]. Fluorescence transients were induced over a leaf area of 4 mm diameter by a red light (peak at 650 nm) of 3000 $\mu molm^{-2}s^{-1}$ (sufficient excitation intensity to ensure closure of all PSII RCs to obtain a true fluorescence intensity of F_M) provided by a high intensity LED array of three light-emitting diodes. A total measuring time of one second was used thought out the experiments [32].

2.4.2 Specific and phenomenological fluxes

Specific activities of active PSII reaction centre *i.e.* antenna size of an active PSII (ABS/RC), electron transport flux from QA to PQ per active PSII (ET/RC) and Dissipated energy flux per reaction centre (DI/RC) and phenomenological fluxes *i.e* Absorption flux per cross section (ABS/CS), Electron transport flux per cross section (ET/CS) and Dissipated energy flux per cross section (DI/CS) were calculated using following equations of JIP test [32].

$$\frac{ABC}{RC} = Mo \cdot \left(\frac{1}{Vj}\right) \cdot \left(\frac{1}{\phi Po}\right) \quad \text{(ii)}$$

$$\frac{TR}{RC} = Mo \cdot \left(\frac{1}{Vj}\right) \quad \text{(iii)}$$

$$\frac{ET}{RC} = Mo \cdot \left[1/\frac{(\mathrm{F2ms}-\mathrm{Fo})}{(\mathrm{Fm}-\mathrm{Fo})}\right] \cdot \Psi o \quad \text{(iv)}$$

$$\frac{ABS}{CS} = Flourescence\ intensity\ at\ 50\mu s(Fo) \quad \text{(v)}$$

$$\frac{TR}{CS} = \phi Po \cdot (ABS/CS) \text{.....(vi)}$$

$$\frac{ET}{CS} = \phi Po \cdot \Psi o \cdot \left(\frac{ABS}{CS}\right) \text{..... (v)}$$

$$ET/CS = \phi Po \cdot \Psi o \cdot (\frac{ABS}{CS}) \text{..... (vi)}$$

Where Mo is approximated initial slope (in ms^{-1}) of the fluorescent transient, calculated as $4 \times (F_{300} - F_0) / (F_M - F_0)$ and Ψ_0 is calculated as 1- V_J. V_j is relative variable fluorescence at the J-step and calculated as $(F_{2ms} - F_0) / (Fm-Fo)$.

2.4.3 Density of active PSII RCs

The concentration of active PSII RCs (RC/CS) was quantified as per the following formula

$$\frac{RC}{CS} = \phi Po \cdot [\frac{Vj}{Mo}] \cdot Fm \text{.....(vii)}$$

2.4.4 Fv/Fm (TR/ABS or φP_o) maximum quantum yield of primary PSII photochemistry

The maximum quantum yield of primary PSII photochemistry was calculated as per Strasser et al. 1995 [32].

$$\phi Po = 1 - (\frac{Fo}{Fm}) \text{.....(vii)}$$

Plant Efficiency Analyzer was used for Chlorophyll *a* fluorescence measurement of after 4 hours of Dye solution treatment on duckweed frond. before measurement, duckweed fronds were dark-adapted for 45-60 min at 26°C. A software Biolyzer v.3.0.6 (developed by Laboratory of Bioenergetics, University of Geneva, Switzerland) was used for analyzing the signals of Chl *a* fluorescence.

Statistical analysis

The data analysis was done using SPSS (v. 21.0) software and the graphs were prepared using Microsoft office. All values presented in the paper are means of three independent replicates. Statistical analyses of data were carried out by ANOVA tests and significant differences were established by Tukey (HSD) tests at $P \leq 0.05$.

2.5 Results and discussion

2.5.1 Effect of amount of duckweed biomass

The initial concentration of dye, temperature , and pH was kept constant in order to make a comparative study for biogenic decolorization of BG4 dye in the presence of deferent amounts of duckweed (2-10 g).The dye removal efficiency was significantly increased with increasing the biomass **(Figure 2a-j)** until it reached a value of 74.72% with the biomass of 6 g. The decolorization of BG4 was spectrophotometrically analyzed, the UV spectra were shown in (**Figure 3**). These results indicate that the duckweed biomass 6 g would be the minimum desired biomass in this study **(Figure 2c and 2h)**. Increasing in duckweed biomass provided more surface area for absorption of the dye molecule [11,12].

2.5.2 Effects of temperature and pH

The temperature is an important environmental factor that alters various biological processes. In the present study, temperature is one of the important and effective parameters. To determine the effect of temperature on biological decolorization was studied at the range of 10-50 ^{o}C at an initial concentration of 30 ml/l. As displayed in **(Figure 4a)**, with increasing the temperature biological decolorization was also increased and the results also showed that the thermal deactivation of dye decolorization was not observed. The reaction of biosorption between

184 duckweed and BG4 was an endothermic process prove through the above finding. The results are
185 similar to literature information that high temperature induces biological dye decolorization
186 capacity [13,33].

187 pH is also one of the major environmental factors and biological decolorization of BG4
188 was highly regulated by pH. In the present study biodecolorization of BG4 was analyzed over a
189 range from 1.0 to 9.0 pH. It was observed that dye decolorization efficiency increases as the pH of
190 the solution increase up to 8 **(Figure 4b).** It can be understood by the concept of zero-point
191 discharge for biomass. An isoelectric point of around 3-4 pH is determined for plant biomass [33,34].
192 The plant surface positively charges in acidic solution and negatively charge in alkaline solution,
193 meanwhile, BG4 is a cationic dye, the high pH solution enhances the bio-adsorption of dye **(Figure**
194 **5)**, thus the dye removal potential increases as reported by Vasanth et al. [21]**.**

195 **2.5.3 FT-IR analysis**

196 **Figure 6** illustrates FT-IR spectra of MG treated by *L. gibba* at different reaction times. FT-IR
197 spectrum of Malachite Green before removal showed the specific peaks in fingerprint region
198 (2500–500 cm^{-1}) and after 6 and 12 h of treatments no significant alteration in fingerprint region
199 was observed which indicate that the mechanism involved in decolorization of MG by duckweed
200 is bio accumulation and not biodegradation.

201 **2.5.4 Enzyme analysis**

202 Plant enzymes play a crucial role in the biodecolorization of pollutants [19] and directly
203 participate in the decolorization of synthetic dyes [35].

204 SOD is an important enzyme of plant antioxidant defense system and it converts two
205 superoxide radicals (O_2) to water and O_2. Subsequently, the products of SOD were furthers

detoxified by other enzymes CAT and GPOD and convert into less toxic compounds [36]. After 24 hours of biodecolorization process, a significant induction (116.67 % and 164.76 %) in SOD activity was observed in *L. gibba* treated with 15 and 30 mg/l of BG4, respectively **(Figure 7a)**. The activity of GPOD and CAT were also increased 120.45 % and 106.96 % respectively from control with exposure by 15 mg/l of BG4. A significant increase was observed in GPOD and CAT activity with exposure with 30 mg/l of BG4 after 24 hours (143.66 % and 113.91 % respectively from control) **(Figure 7b and c)**. As displayed in Figure 6 antioxidant enzyme activity increases significantly ($p \leq 0.05$). The presence of pollutants in the environment around the plants leads to oxidative stress and produces a high amount of reactive oxygen species (ROS) and perhaps these antioxidant enzymes directly involved in the conversion of these harmful ROS into a less toxic product [37]. The high concentration of dye leads to an increased amount of ROS [12] and for protecting the plant from these deleterious components antioxidant systems become activate [36]. SOD, GPOD, and CAT are major components of the antioxidant system of the plant [35]

2.5.5 Recyclability of live L. gibba

To examine the reusability of *L. gibba* in Basic Green 4 decolorization a repeated batch operation was performed. During five repeated batch run it was observed that *L. gibba* showed equal dye decolorization efficiency **(Figure 8)**. From these results, we can conclude that *L. gibba* possesses a great ability to recycle or reusability in repetitive decolorization processes. The results also indicate that the treatment of the BG4 solution by the duckweed is a biological decolorization process. A similar result was also reported for biodecolorization of dye AB92 by *L. minor* [38], BG4 by algae *Chara* sp.[20].

2.5.6 Photosynthetic performance

During dye decolorization, L. *gibba* exhibited no profound effect on photosynthetic efficiency. However, the activities of specific fluxes (ABS/RC, TR/RC, and ET/RC) were found more sensitive to BG4 **(Table 4)**. The BG4-induced decline in ABS/RC and TR/RC 19.19 % and 17.96% respectively. Similarly, the ET/RC reduced 9.62% during the complete decolorization of BG4. To compensate for the BG4-induced reduction in specific fluxes, the plants increased the RC/CS. The density of active RCs increased by 6.88% in BG4-treated plants as compared to controls **(Figure 9a).** Transformation of inactive PSII RCs into active form displays physiological adaptation in *L. gibba* against BG4-induced chemical stress [31].

BG4 treated plants exhibited a slight reduction in light-harvesting and trapping efficiencies per cross-section (ABS/CS and TR/CS) when matched with controls. The values of ABS/CS and TR/CS decreased 10.98 and 9.83 in plants subjected to BG4 decolorization. Reduction in ABS/CS and TR/CS may be due to the decline in ABS/RC and TR/RC [39].

Overall electron transport rate per cross-section (ET/CS) remained almost unchanged in BG4 treated plants, which indicates that *L. gibba* enhanced the concentrations of active PSII RCs to maintain the rate of ET/CS. Similarly. no significant variations in Fv/Fm were observed during the entire duration (12h) of BG4 decolorization, which indicates that *L. gibba* has high potential to maintain its photosynthetic efficiency even during/after the decolorization of BG4 by modulating the specific, phenomenological fluxes, the density of active PSII RCs and Fv/Fm **(Figure 9b).** Chlorophyll fluorescence analysis demonstrates that *L. gibba* has high physiological adaption to sustain overall photosynthesis during the post-decolorization of BG4.

Conclusion

The results from present research work give a positive sign that *L. gibba* has remarkable potential for decolorization of BG4. The *L. gibba* mediated BG4 decolorization depends on various parameters that are assessed in this study. As increasing pH, Temperature, contact time, and plant weight the BG4 decolorization capacity was also increased. The study revealed that the temperature at 25-30 ^{o}C and pH 8.0 are considered as optimum for the best results. BG4 treatment to *L. gibba* leads to activation of antioxidant activity which determined by the increased value of SOD, CAT, and GPOD which usually activated when plants suffering unfavorable environmental conditions. The repeated batch experiment confirms the reusability of *L. gibba* for BG4 decolorization.

The study of chlorophyll fluorescence also reveals that photosynthetic apparatus of *L. gibba* is highly tolerant of BG4 and did not alter even during and after the dye decolorization. The overall results of the present findings highlight that duckweed *L. gibba* can be used as a potent organism for biodecolorization of BG4.

Declarations

Funding:

This research did not receive any specific grant funding from agencies in the public, commercial, or not-for-profit sectors.

Conflicts of interest/Competing interests: The authors declare no competing interest.

Ethics approval: None

Authors' contributions:

HS conceived the idea and designed the research plan. HS, SR, DK, SS, UB and VS execute the experiment. HS performed writing – original draft and conceptualization, data analysis and prepare all figures and artwork. VS supervised the complete work. All the authors contributed to discussing

and reviewing the manuscript. Finally, all the authors read and approved the final version of the manuscript for publication.

Acknowledgment

The authors thank the Mohanlal Sukhadia University, India for providing laboratory facilities. Authors are also grateful to Prof. Reto J. Strasser, University of Geneva, Switzerland for his help in the analysis of chlorophyll fluorescence data.

References

1. Imron, M. F., Kurniawan, S. B., Soegianto, A. & Wahyudianto, F. E. Phytoremediation of methylene blue using duckweed (Lemna minor). *Heliyon* (2019) doi:10.1016/j.heliyon.2019.e02206.

2. Shanmugam, L., Ahire, M. & Nikam, T. Bacopa monnieri (L.) Pennell, a potential plant species for degradation of textile azo dyes. *Environ. Sci. Pollut. Res.* (2020) doi:10.1007/s11356-019-07430-x.

3. Mohan, S. V, Bhaskar, Y. V & Karthikeyan, J. Biological decolourisation of simulated azo dye in aqueous phase by algae Spirogyra species. *Int. J. Environ. Pollut.* (2004) doi:10.1504/IJEP.2004.004190.

4. Rodríguez Couto, S. Dye removal by immobilised fungi. *Biotechnol. Adv.* **27**, 227–235 (2009).

5. Kiliç, N. K., Duygu, E. & Dönmez, G. Triacontanol hormone stimulates population, growth and Brilliant Blue R dye removal by common duckweed from culture media. *J. Hazard. Mater.* (2010) doi:10.1016/j.jhazmat.2010.06.063.

6. Aksu, Z. & Dönmez, G. Combined effects of molasses sucrose and reactive dye on the growth and dye bioaccumulation properties of Candida tropicalis. *Process Biochem.* **40**, 2443–2454 (2005).

7. Walsh, G. E., Bahner, L. H. & Horning, W. B. Toxicity of textile mill effluents to freshwater and estuarine algae, crustaceans and fishes. *Environ. Pollution. Ser. A, Ecol. Biol.* (1980)

298 doi:10.1016/0143-1471(80)90161-0.

299 8. Raj, S., Singh, H., Trivedi, R. & Soni, V. Biogenic synthesis of AgNPs employing
300 Terminalia arjuna leaf extract and its efficacy towards catalytic degradation of organic dyes.
301 *Sci. Rep.* **10**, 9616 (2020).

302 9. Kivaisi, A. K. The potential for constructed wetlands for wastewater treatment and reuse in
303 developing countries: a review. *Ecol. Eng.* **16**, 545–560 (2001).

304 10. Ertuğrul, S., San, N. O. & Dönmez, G. Treatment of dye (Remazol Blue) and heavy metals
305 using yeast cells with the purpose of managing polluted textile wastewaters. *Ecol. Eng.* **35**,
306 128–134 (2009).

307 11. Kaushik, P. & Malik, A. Fungal dye decolourization: Recent advances and future potential.
308 *Environ. Int.* **35**, 127–141 (2009).

309 12. Khataee, A. R., Pourhassan, M. & Ayazloo, M. Biological decolorization of C.I. basic green
310 4 solution by chlorella sp.: Effect of operational parameters. *Chinese J. Appl. Environ. Biol.*
311 (2009) doi:10.3724/SP.J.1145.2009.00110.

312 13. Daneshvar, N., Ayazloo, M., Khataee, A. R. & Pourhassan, M. Biological decolorization of
313 dye solution containing Malachite Green by microalgae Cosmarium sp. *Bioresour. Technol.*
314 **98**, 1176–1182 (2007).

315 14. Banat, I. M., Nigam, P., Singh, D. & Marchant, R. Microbial decolorization of textile-
316 dyecontaining effluents: A review. *Bioresour. Technol.* **58**, 217–227 (1996).

317 15. Jinqi, L. & Houtian, L. Degradation of azo dyes by algae. *Environ. Pollut.* (1992)
318 doi:10.1016/0269-7491(92)90127-V.

319 16. Pilon-Smits, E. PHYTOREMEDIATION. *Annu. Rev. Plant Biol.* **56**, 15–39 (2005).

320 17. Saranya, G., Saravanan, P., Dharmendira Kumar, M. & Renganathan, S. Equilibrium
321 Uptake and Bioaccumulation of Basic Violet 14 Using Submerged Macrophyte Hydrilla
322 verticillata. *Clean - Soil, Air, Water* (2011) doi:10.1002/clen.201000186.

323 18. Susarla, S., Medina, V. F. & McCutcheon, S. C. Phytoremediation: An ecological solution
324 to organic chemical contamination. in *Ecological Engineering* (2002). doi:10.1016/S0925-

8574(02)00026-5.

19. Aubert, S. & Schwitzguébel, J.-P. Screening of plant species for the phytotreatment of wastewater containing sulphonated anthraquinones. *Water Res.* **38**, 3569–3575 (2004).

20. Khataee, A. R., Dehghan, G., Ebadi, A., Zarei, M. & Pourhassan, M. Biological treatment of a dye solution by Macroalgae Chara sp.: Effect of operational parameters, intermediates identification and artificial neural network modeling. *Bioresour. Technol.* (2010) doi:10.1016/j.biortech.2009.11.079.

21. Vasanth Kumar, K., Sivanesan, S. & Ramamurthi, V. Adsorption of malachite green onto Pithophora sp., a fresh water algae: Equilibrium and kinetic modelling. *Process Biochem.* (2005) doi:10.1016/j.procbio.2005.01.007.

22. Parshetti, G., Kalme, S., Saratale, G. & Govindwar, S. Biodegradation of malachite green by Kocuria rosea MTCC 1532. *Acta Chim. Slov.* (2006).

23. Kannan, C., Sundaram, T. & Palvannan, T. Environmentally stable adsorbent of tetrahedral silica and non-tetrahedral alumina for removal and recovery of malachite green dye from aqueous solution. *J. Hazard. Mater.* (2008) doi:10.1016/j.jhazmat.2007.12.116.

24. Wang, W. Literature review on duckweed toxicity testing. *Environ. Res.* (1990) doi:10.1016/S0013-9351(05)80147-1.

25. Stomp, A.-M. The duckweeds: A valuable plant for biomanufacturing. in vol. 11 69–99 (Elsevier, 2005).

26. Radić, S., Babić, M., Škobić, D., Roje, V. & Pevalek-Kozlina, B. Ecotoxicological effects of aluminum and zinc on growth and antioxidants in Lemna minor L. *Ecotoxicol. Environ. Saf.* (2010) doi:https://doi.org/10.1016/j.ecoenv.2009.10.014.

27. OECD. Lemna sp. Growth Inhibition Test. *Oecd Guidel. Test. Chem.* 22 (2002).

28. Kono, Y. Generation of superoxide radical during autoxidation of hydroxylamine and an assay for superoxide dismutase. *Arch. Biochem. Biophys.* **186**, 189–195 (1978).

29. Teranishi, Y., Tanaka, A., Osumi, M. & Fukui, S. Catalase activities of hydrocarbon-utilizing candida yeasts. *Agric. Biol. Chem.* (1974) doi:10.1080/00021369.1974.10861301.

30. Racusen, D. & Foote, M. Protein synthesis in dark-grown bean leaves. *Can. J. Bot.* **43**, 817–824 (1965).

31. Wungrampha, S. *et al.* CO2 uptake and chlorophyll a fluorescence of Suaeda fruticosa grown under diurnal rhythm and after transfer to continuous dark. *Photosynth. Res.* (2019) doi:10.1007/s11120-019-00659-0.

32. Strasser, R. J., Srivastava, A. & Govindjee. Polyphasic chlorophyll a fluorescence transient in plants and cyanobacteria. *Photochem. Photobiol.* (1995) doi:10.1111/j.1751-1097.1995.tb09240.x.

33. Çetinkaya Dönmez, G., Aksu, Z., Öztürk, A. & Kutsal, T. A comparative study on heavy metal biosorption characteristics of some algae. *Process Biochem.* **34**, 885–892 (1999).

34. Crist, R. H., Oberholser, K., Shank, N. & Ming Nguyen. Nature of bonding between metallic ions and algal cell walls. *Environ. Sci. Technol.* **15**, 1212–1217 (1981).

35. Schröder, P., Daubner, D., Maier, H., Neustifter, J. & Debus, R. Phytoremediation of organic xenobiotics - Glutathione dependent detoxification in Phragmites plants from European treatment sites. *Bioresour. Technol.* (2008) doi:10.1016/j.biortech.2007.12.081.

36. Singh, H., Kumar, D. & Soni, V. Copper and mercury induced oxidative stresses and antioxidant responses of Spirodela polyrhiza (L.) Schleid. *Biochem. Biophys. Reports* **23**, 100781 (2020).

37. Chen, J. *et al.* Copper induced oxidative stresses, antioxidant responses and phytoremediation potential of Moso bamboo (Phyllostachys pubescens). *Sci. Rep.* **5**, 1–9 (2015).

38. Khataee, A. R., Movafeghi, A., Torbati, S., Salehi Lisar, S. Y. & Zarei, M. Phytoremediation potential of duckweed (Lemna minor L.) in degradation of C.I. Acid Blue 92: Artificial neural network modeling. *Ecotoxicol. Environ. Saf.* (2012) doi:10.1016/j.ecoenv.2012.03.021.

39. Kumar, D., Singh, H., Raj, S. & Soni, V. Chlorophyll a fluorescence kinetics of mung bean (Vigna radiata L.) grown under artificial continuous light. *Biochem. Biophys. Reports* **24**, 100813 (2020).

Figure Legends

Figure 1 Macroscopic view of fronds and rhizoids of *L. gibba* (a) and Physical observation of *L. gibba*-mediated decolorization (b)

Figure 2 The absorbance of BG4 of 2g (a), 4g (b), 6g (c), 8g (d) and 10g (e) after water contaminated with 30 mg/l of BG4 dye was treated with *L. gibba* for 12 hours. Percent removal and concentration of BG4 (f-j) respectively. Values are presented in the average of triplicates ±SD. Different characters indicate significant differences among the results ($p \leq 0.05$).

Figure 3 UV spectra of BG4 (30 mg/l) biodegraded by *L. gibba* at time 0 – 12 hrs.

Figure 4 Effect of different temperature on biodecolorization of BG4 (pH= 8.0, Plant weight= 4 g, $[BG4]_0$= 30 mg/l, (a) Effect of pH of dye solution on biodecolorization of BG4 (T= 30 ^oC, Plant weight = 6 g, Time 7 h) (b).

Figure 5 Graphical presentation of mechanism of *L. gibba* mediated decolorization of BG4.

Figure 6 FT-IR spectra of MG (10 mg/l) biodegraded by *L. gibba* at times: 0,6 and 12 hours (T = 30 ^oC, pH 8, Plant weight = 4 g).

Figure 7 activities of SOD (a) CAT (b) GPOD (c) in control and treated *L gibba* with 15 and 30 mg/l BG4. Values are presented in the average of triplicates ±SD. Different characters indicate significant differences among the results ($p \leq 0.05$).

Figure 8 Biological decolorization profile during repeated-batch operation (T= 30^oC, pH= 8.0, $[BG4]_0$= 30mg/l, Plant weight = 6g).

Figure 9 Radar plot showing the specific, phenomenological, and Fv/Fm before and after maximum decolorization of BG4 in *L. gibba* (a). Specific membrane models and phenomenological yield models representing the changes in various photosynthetic parameters in control and BG4 treated *L. gibba* (b).

Figures

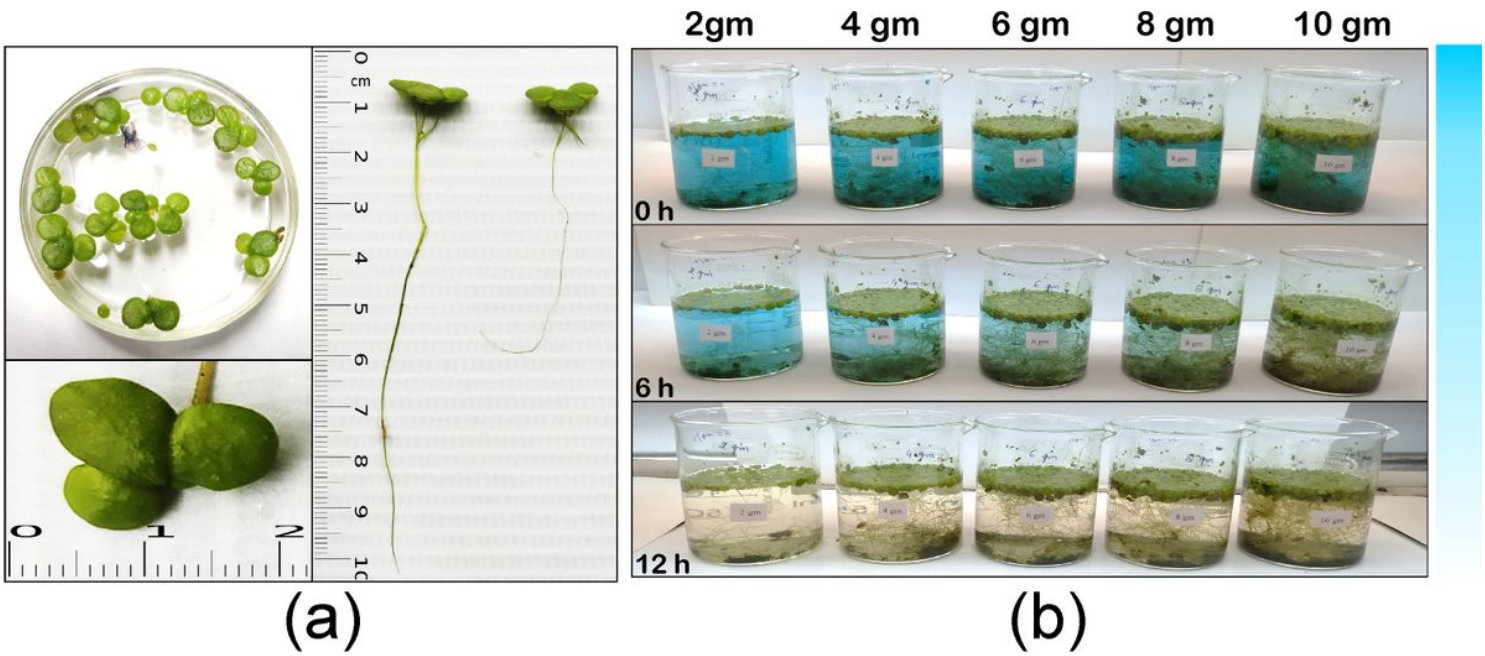

Figure 1

Macroscopic view of fronds and rhizoids of L. gibba (a) and Physical observation of L. gibba-mediated decolorization (b)

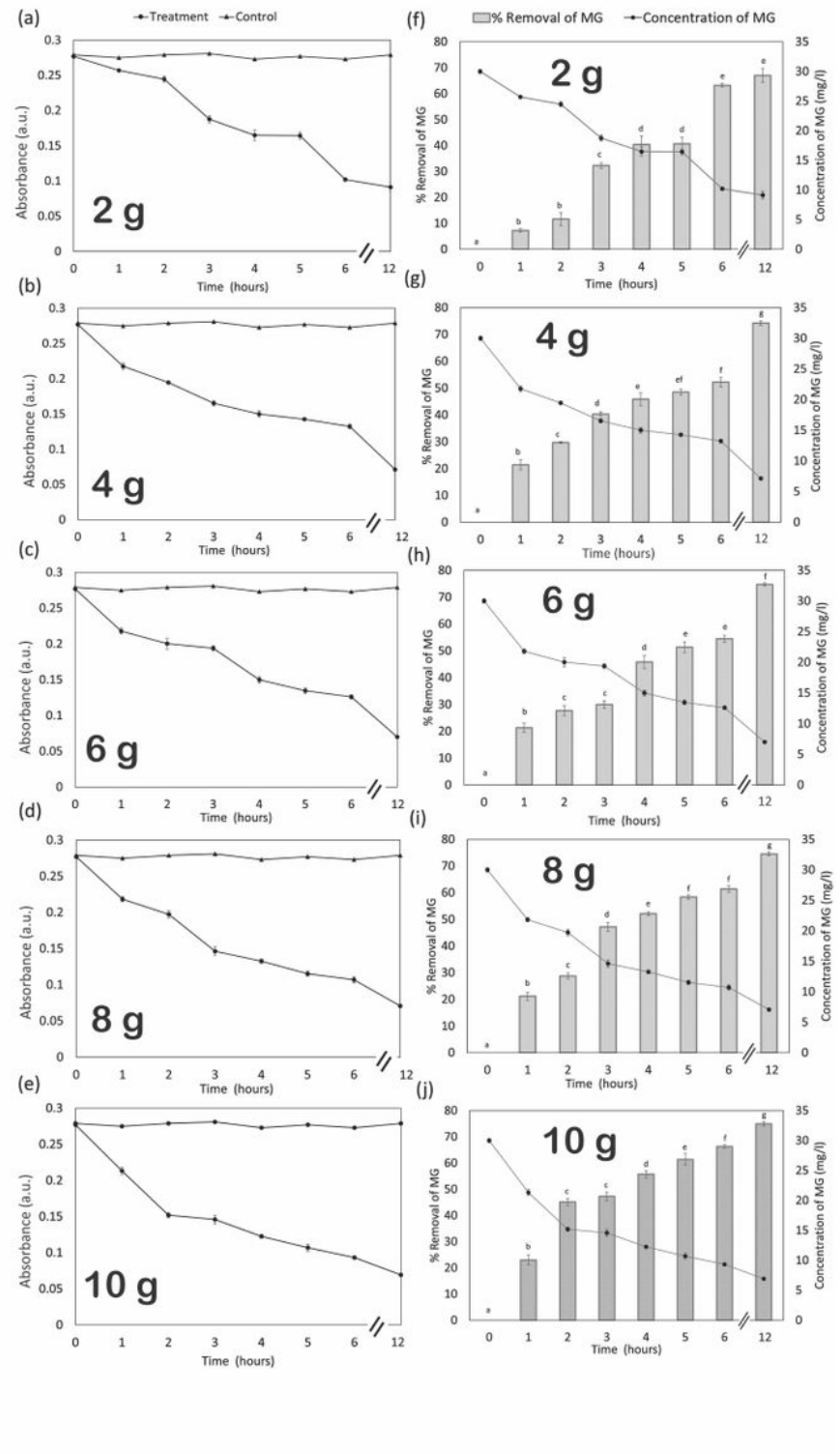

Figure 2

The absorbance of BG4 of 2g (a), 4g (b), 6g (c), 8g (d) and 10g (e) after water contaminated with 30 mg/l of BG4 dye was treated with L. gibba for 12 hours. Percent removal and concentration of BG4 (f-j) respectively. Values are presented in the average of triplicates ±SD. Different characters indicate significant differences among the results ($p \leq 0.05$).

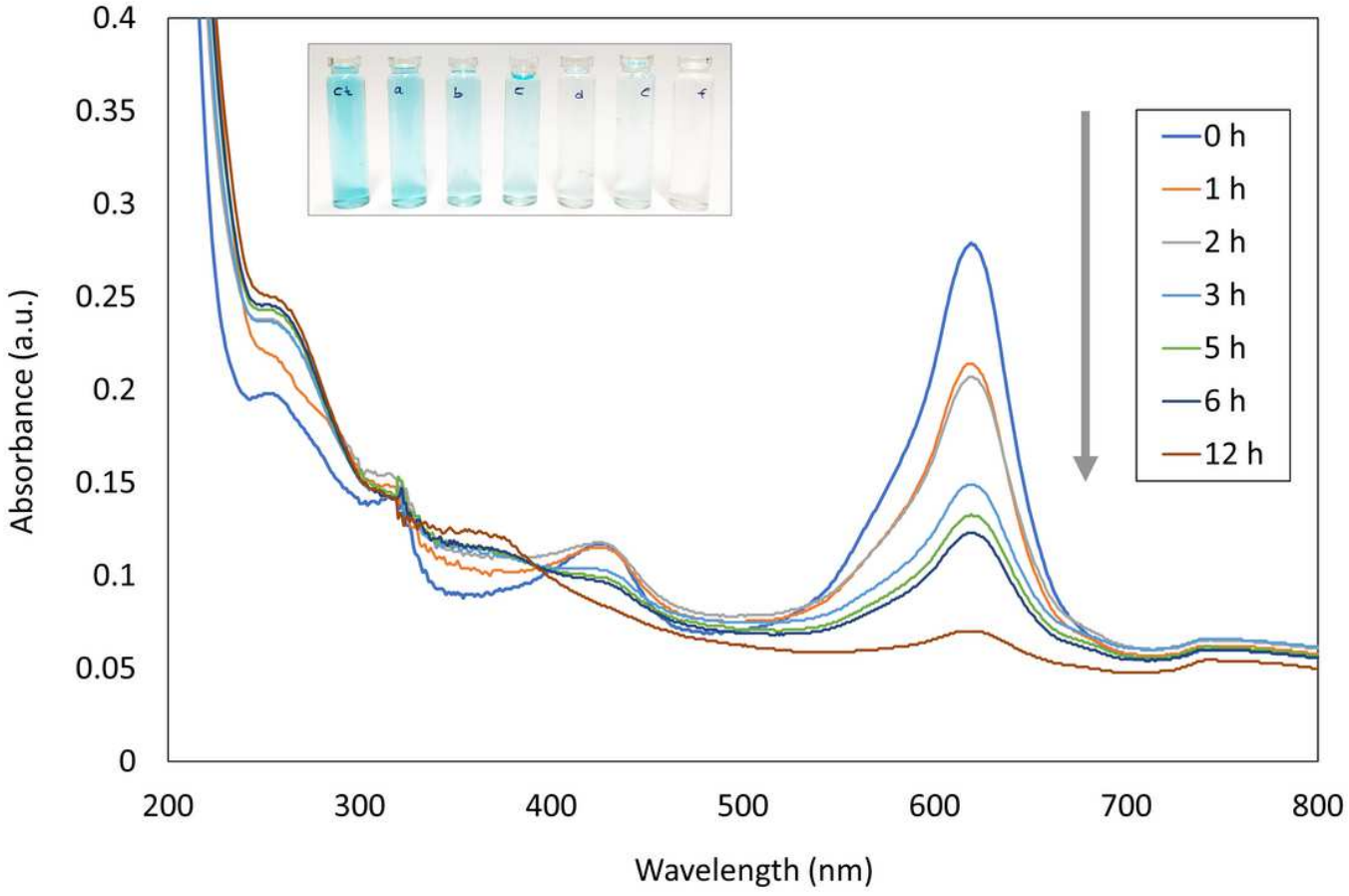

Figure 3

UV spectra of BG4 (30 mg/l) biodegraded by L. gibba at time 0 – 12 hrs.

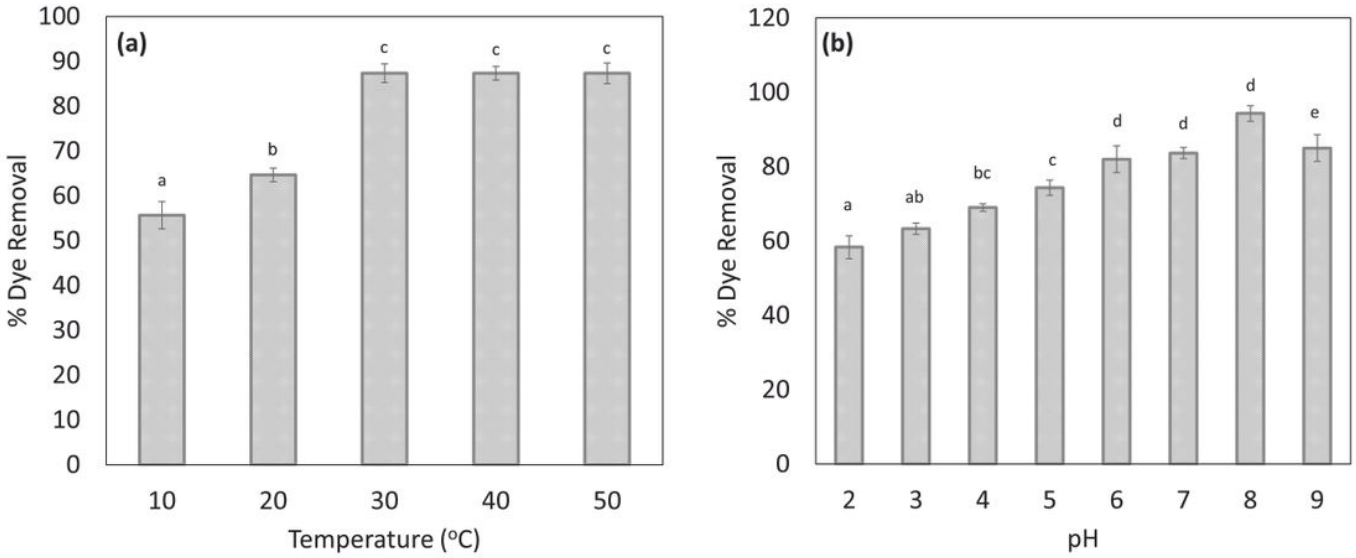

Figure 4

Effect of different temperature on biodecolorization of BG4 (pH= 8.0, Plant weight= 4 g, [BG4]0= 30 mg/l, (a) Effect of pH of dye solution on biodecolorization of BG4 (T= 30 oC, Plant weight = 6 g, Time 7 h) (b).

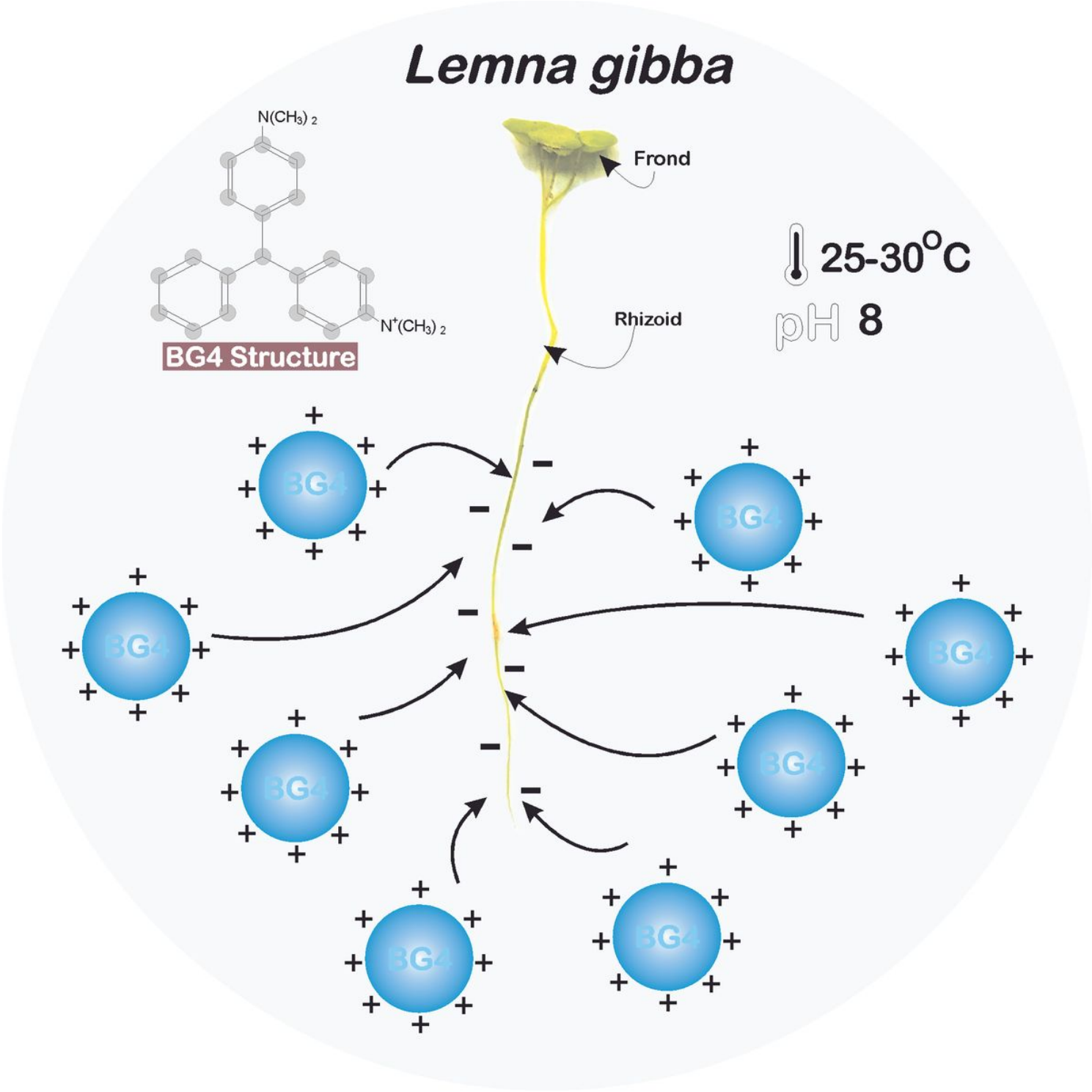

Figure 5

Graphical presentation of mechanism of L. gibba mediated decolorization of BG4.

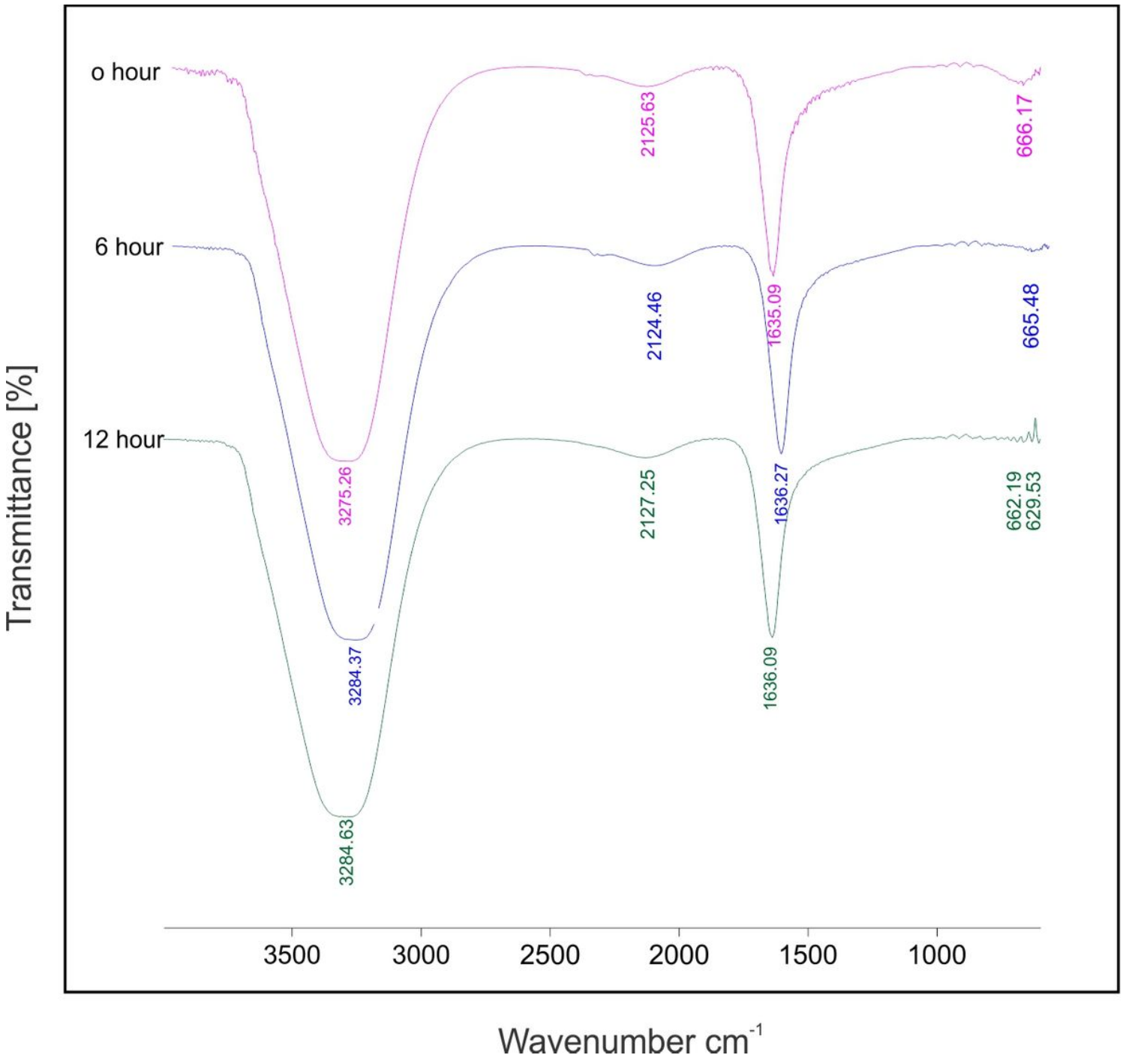

Figure 6

FT-IR spectra of MG (10 mg/l) biodegraded by L. gibba at times: 0,6 and 12 hours (T = 30 oC, pH 8, Plant weight = 4 g).

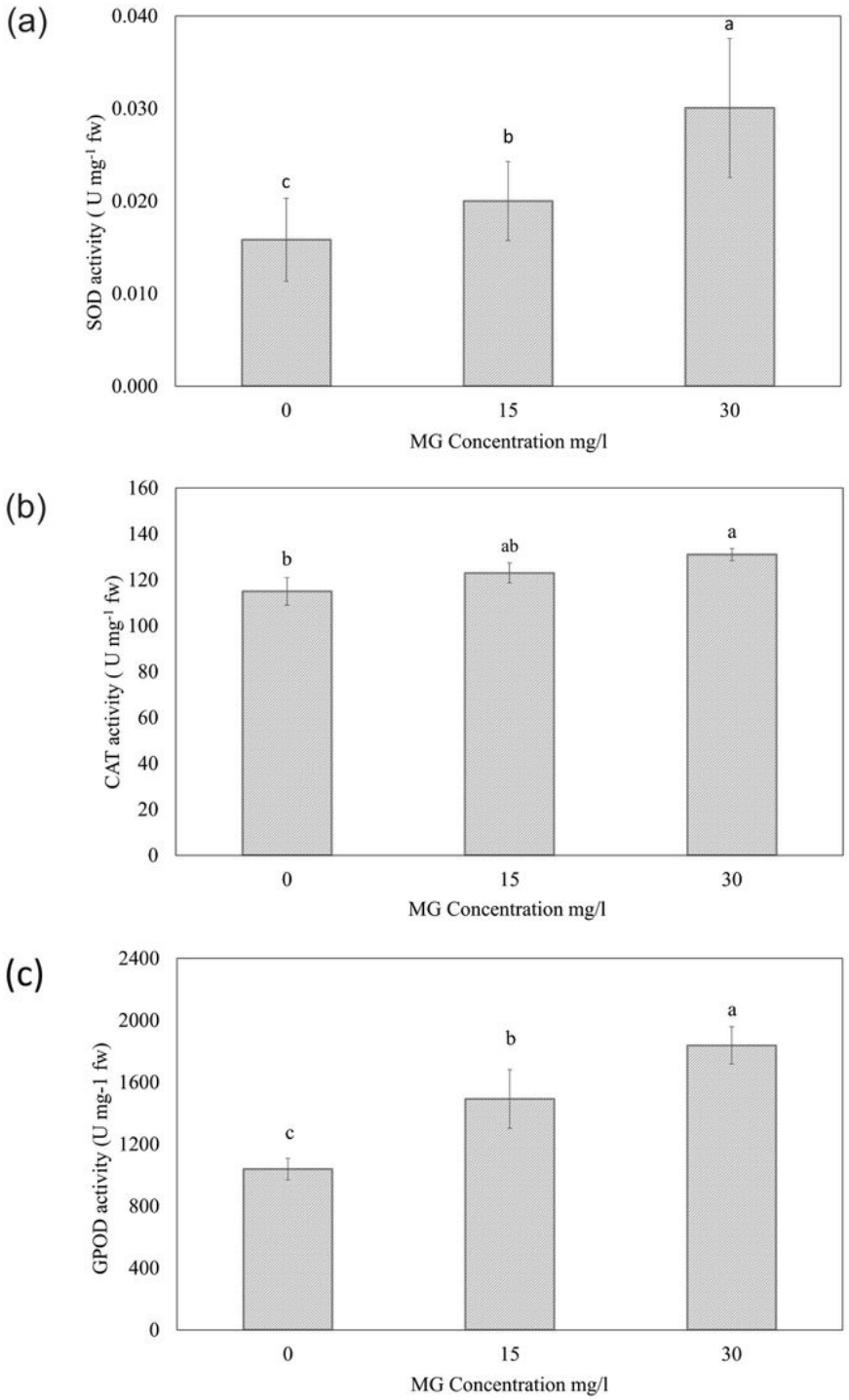

Figure 7

activities of SOD (a) CAT (b) GPOD (c) in control and treated L gibba with 15 and 30 mg/l BG4. Values are presented in the average of triplicates ±SD. Different characters indicate significant differences among the results ($p \leq 0.05$).

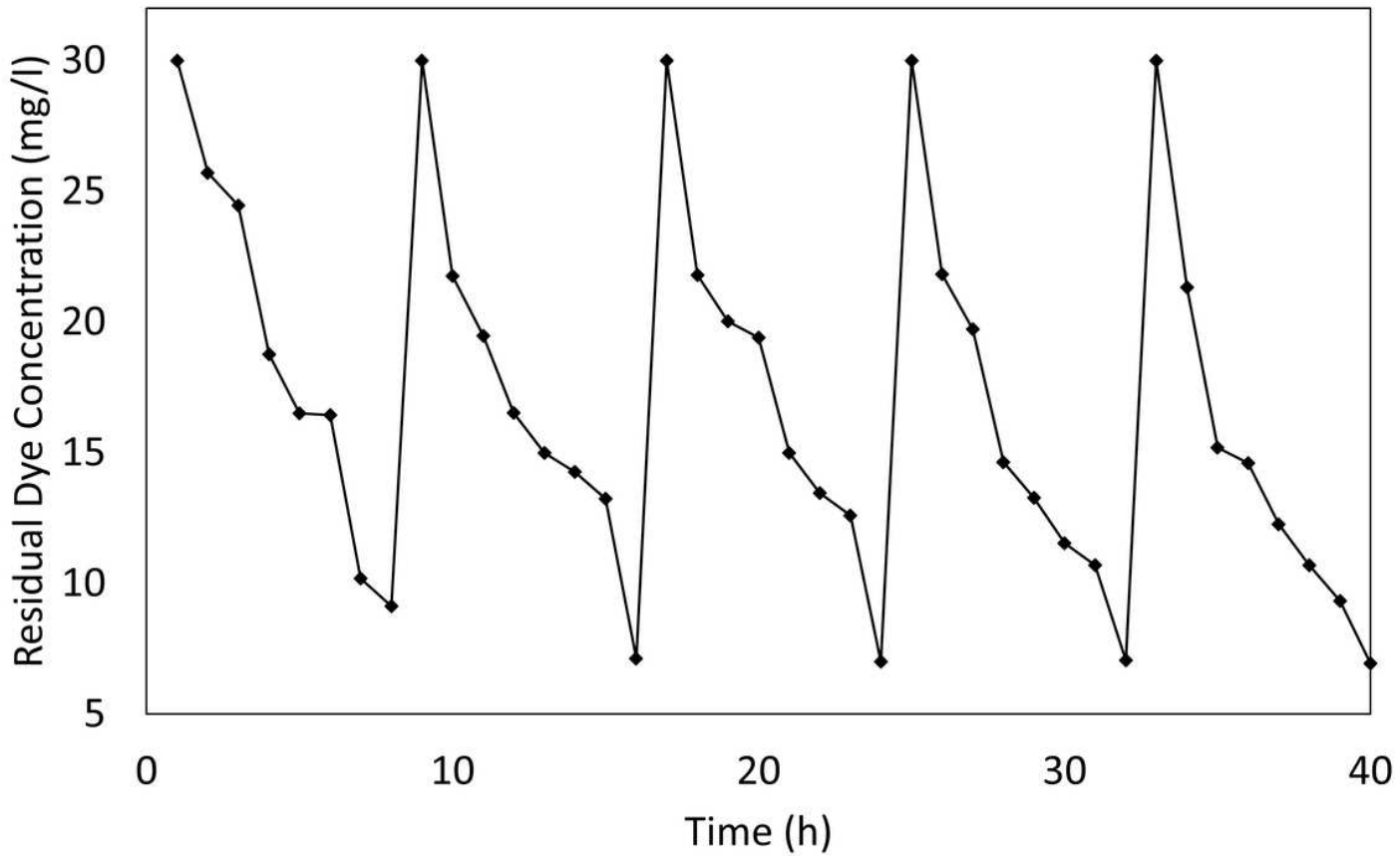

Figure 8

Biological decolorization profile during repeated-batch operation (T= 30oC, pH= 8.0, [BG4]0= 30mg/l, Plant weight = 6g).

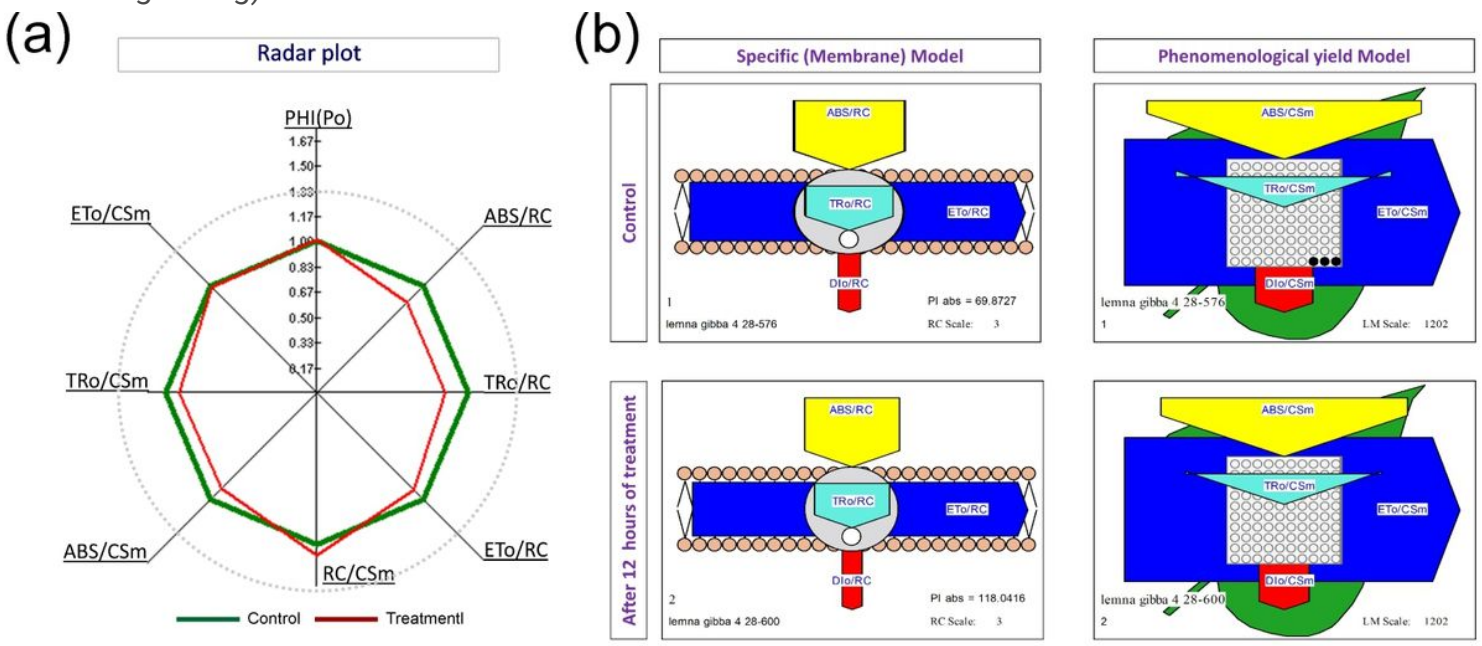

Figure 9

Radar plot showing the specific, phenomenological, and Fv/Fm before and after maximum decolorization of BG4 in L. gibba (a). Specific membrane models and phenomenological yield models representing the changes in various photosynthetic parameters in control and BG4 treated L. gibba (b).

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.docx
- Table2.docx
- Table3.docx
- Table4.docx
- GraphicalAbstract.jpg