Environment and Ecology39 (4A) : 1422—1433, October—December 2021
ISSN 0970-0420

Degradation and Decolorization of Malachite Green Dye by *Bacillus subtilis* Isolated From Dye Effluent Sample

Alka Rani, Padma Singh

Received 6 August 2021, Accepted 12 September 2021, Published on 4 October 2021

ABSTRACT

The increase usage of dyes causes increasing concentration of dyes in the environment it becomes absolutely necessary to develop more efficient and cost- effective ways to remove dyes from effluents prior to releasing them into the environment in order to nullify or at least minimize their harmful effects. In this study effluent sample of industry was collected from the adjoining area of Haridwar. In triphenylmethane dyes are being used extensively in various industries. In this experiment the potent bacterial isolate 4DD15 was isolated from the Common Effluent of SIDCUL industries of Haridwar. On the basis of 16S rRNA identification 4DD15 was identified as a *Bacillus subtilis*. Strain 4DD15 has been decolorized 95.03% malachite green dye in 72h at 37°C. Different growth parameters have also been optimized for the decolorization. Highest decolorization percentage was observed at a temperature 37^{0}C, pH 7.0 under the shaking and aerobic condition, sucrose was found to be the best carbon source and peptone was found best nitrogen source for malachite green decolorization. Bacterial strain 4DD15 showed positive results for the decolorization of the dyes under aerobic condition. Phytotoxicity of dye and degraded dye evaluate by *in vitro* (Plate assay) and *in vivo* (Pot assay) and observed that dye was drastically affected the seed germination percentage and seedling growth.

Keywords Malachite green, Dye, Decolorization, Bacteria, *Bacillus subtilis.*

Alka Rani, Padma Singh
Department of Microbiology, Kanya Gurukul Campus, Gurukul Kangri University, Haridwar (Uttarakhand) 249407, India
Email : alkamicro90@gmail.com
*Corresponding author

INTRODUCTION

Industrial effluent is caused the serious environmental problem. Every industry like textile, paper, leather, cosmetic, food and plastic industries using the different dyes as a coloring effluent of these industries contains synthetic dyes that are toxic, mutagenic and carcinogenic (Ajaz *et al*. 2020). Malachite green is widely used dye of triphenylmethane group. Due to its low cost, ready availability and high efficacy against fish microbial pathogens mostly used in the textile industry and the fish farming industry (Qi *et al*. 2020, Riegas-Villalobos *et al.* 2020). Malachite green dye is easily available and low cost dye despite of this malachite green is highly toxic and has been banned by the US food and drug administration (Ali *et al.* 2020, Al-Tohamy *et al.* 2020). Because of toxic nature of malachite green various cost effective methods have

been explored for decontaminate waste water and protect the environment (Amiri *et al*. 2020). Developing microbial biodegradation agents is an important approach for solving these problems due to their effective cost, friendliness to the environment and small quantities of sludge. Despite of this so many bacteria are decolorized industrial dyes, but only few are able to degrade these compounds into CO_2 and H_2O (An *et al.* 2020). Industrial dyes are broken down into an aromatic amine and arylamine to be carcinogenic and mutagenic these compound reduce seed germination rate and seedling growth. Different microorganisms like bacteria, fungi and algae capable of decolorizing a wide range of dyes with high efficiency (Atallah *et al.* 2019). Microbial decolorization and degradation is ecofriendly and cost-competitive alternative to other physical and chemical decontamination methods (Awad *et al*. 2019, Unuofin *et al.* 2019). The growth of plants irrigated with dye containing industrial effluent significantly affected. It was reported that *Bacillus* sp., *Chlorella* sp., *Shewanella* sp., *Pseudomonas* sp. have potential to degrade industrial dye (Upadhyay *et al.* 2020, Pourrahim *et al*. 2020). The main objective of present study was to identified the potent decolorizing bacteria and its optimized condition for the high rate of decolorization and molecular characterization.

MATERIALS AND METHODS

Sampling

The effluent samples were collected from common effluent of SIDCUL industries of Haridwar. Standard procedures were followed during sampling. The temperature and pH were determined at the sampling site. The pH was determined by using pH strip and temperature with laboratory thermometer. The sample were transported to laboratory at 4°C as in accordance with the standard methods.

Dyes and culture media

Malachite green (MG), peptone, sodium chloride, beef extract, yeast extract and agar powder were obtained from Hi-Media laboratory, India. All chemicals were of highest purity and analytical grade.

Isolation and screening

Isolation were carried out by serially diluting industrial effluent in sterile distilled water. Serial dilutions from 10-1 to 10-7 were prepared by pipette out the 1 ml of dye effluent sample in to distilled water blank. The nutrient agar plates were prepared and labeled, then 0.1 ml of aliquot from 10-6 and 10-7 was spread on agar plate using the spread plate technique and incubated at 37^0C for 24 h (Waksman 1961).

Identification of selected isolates

The selected isolate 4DD15 was examined for the morphological properties, such as size, shape, cell arrangement and staining properties. Biochemical characteristics of the isolate was evaluated. The isolate 4DD15 was identified up to species based on comparative analysis of the observed characteristics with the standard description of bacterial strains in Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons 1974).

Visual identification of decolorization

15 morphologically distinct bacterial isolates were screened for their ability to decolorize dye namely malachite green. Test tubes containing 10 ml of nutrient broth medium with 100 mg/L of malachite green. The tubes were inoculated with loopful culture of morphologically different isolated bacterial strain. The tubes were incubated for 72h under static condition at 37°C±2. After 72h incubation the tubes were observed for decolorization and was ranked on the basis of visual identification as intense (+++), moderate (++), slight (+) and no decolorization (-). The screened culture was transfer to agar slant and store 4°C for further study. Isolate 4DD15 showed best decolorization of the added dye. 4DD15 efficient bacterial strains were selected for further studies (Barathi and Arulselvi 2015).

Dye decolorization assay

100 ml of sterile nutrient broth was prepared in 250 ml Erlenmeyer flask and malachite green was amended at a concentration of 200 mg/L^{-1}. The flask was then inoculated with 1 % of *Bacillus subtilis* culture suspension and incubated at 37 ºC for 24h. After incubation at defined intervals of 24h, 48h and 72h the culture was withdrawn, centrifuged at

10,000 g and 4^0C for 20 min and decolorization was detrimined by measuring the optical density of the cell free medium with the help of spectrophotometer at wavelength maxima 620 nm for malachite green. The uninoculated dye free medium was used as blank (Telke *et al.* 2010).

$$\text{Decolorization percentage} = \frac{\text{Initial abdorbance} - \text{Observed absorbance}}{\text{Initial absorbance}} \times 100$$

Optimization

Several parameters were optimized to know the favorable condition for highest decolorization of malachite green (Shah *et al.* 2013).

Temperature

Decolorization of malachite green by the best potent strain 4DD15 was studied at different temperatures such as 25, 37, 40 and 45°C and incubated for 120h. Decolorization was determined on the different time interval by measuring the absorbance at the 620 nm for malachite green by using spectrophotometer.

pH

Effect of different pH ranging from 5 to 9 was examined on the decolorization efficiency of the selected isolates. Incubation temperature was maintained at 37°C. The experiment was conducted in triplicate. Decolorization was determined in the form of decolorization percentage.

Carbon source

To determine the effects of different carbon sources on bacterial decolorization, seven carbon sources, namely, maltose, sorbitol, lactose, mannitol, fructose, dextrose and sucrose were used at the rate of 5 g L^{-1}. The 24 h incubated inoculum was added to the medium then incubated at 37°C for 120h.

Effect of various nitrogen sources

The effect of various nitrogen sources viz., Yeast extract, Beef extract, Ammonium sulfate and Peptone was analyzed in this present study. The nitrogen sources 5 g/L were added in the nutrient broth containing malachite green and incubated at 37°C for 120h in a rotary shaker running at 120 rpm. Decolorization assay was measured in the terms of percentage.

Effect of different conditions

The dye decolorization was measured in different conditions static condition and shaking condition. The cultures were incubated at 37°C for 120h in a static condition and rotary shaker running at 120 rpm. Decolorization assay was measured in the terms of percentage decolorization using spectrophotometer.

Extraction of metabolites

The decolorized sample was centrifuged at 10,000 rpm for 20 min after decolorization of dyes. The supernatant obtained was used to equal volume of ethyl acetate used for extraction of metabolites. The extract was dry over anhydrous sodium sulfate and evaporates it. The metabolite obtained after evaporation were used for phytotoxicity analysis (Amin and Kassem 2012).

Phytotoxicity assessment

Phytotoxicity assessment was done to determine the toxic nature of dye and degraded dye. In the following experiment, untreated dye indicates colored dye or the dye that was not treated with the efficient bacterium and treated dye indicates decolorized dye or the dye that was decolorized by the most efficient bacteria (Gomare *et al.* 2009).

Seed germination study (Plate assay)

The experiment was done in petri plates. The bottom of the petri plates were padded with a layer of whatman paper. Chickpea (*Cicer arietinum*) seeds were soaked for 12 h in 25 ml of colored/untreated dye and 25 ml of decolorized/treated dye simultaneously a control set with the plain water supply was carried out. After soaking 10 seeds were placed in each petri plates. The plates were incubated for 7 days. The tissue paper was kept moist by spraying dye mixture and water for control. After 3 days the germination percentage was calculated using the following formula. After complete germination, the length of plumule of germinated seeds was measured (Verma *et al*. 2017).

$$\text{Seed germination \%} = \frac{\text{Number of seeds germinated in the extract}}{\text{Number of seeds germinated in the dye control}} \times 100$$

Seedling growth study (Field assay)

For assessed the phytotoxicity of dye on the different parameter of plant experiments were carried out in field. Chickpea seeds from every treatment were added to the soil. Dye solution was used for irrigating the seeds separately. The seeds irrigated with distilled water were taken as the control. After 21 days, crop plant from the field study experiment was used to calculate total carbohydrate, total protein and total chlorophyll content.

Effect of dye on total chlorophyll, total carbohydrate and total protein of plant

Total chlorophyll

Chlorophyll content of chickpea leaves was measured by the method as described by Lictenthaler and Wellburn (1994), with some modifications. About 0.3 g of leaves were taken and crushed in 3 ml 80% chilled acetone. Then crushed sample were transferred in a fresh eppendorf tubes and centrifuge at 10,000 rpm for 10 min. The supernatant was taken in clean test tube then absorbance was measured at 663 nm, 645 nm and 510 nm by using visible spectrophotometer.

$$\text{Total chlorophyll (mg/g)} = \frac{(20.2 \times A_{645} + 8.02 \times A_{663}}{1000 \times W} \times V$$

Carotenoid content

Extracted extract for chlorophyll content estimation was used for estimation of carotenoid content of the leaves. Absorbance was measured at 470 nm by using visible spectrophotometer (Kirk and Allen 1965). Carotenoid content of leaf extract was calculated using the given formula.

$$\text{Carotenoid (mg/ g)} = \frac{[(A480 + 0.11 \times A_{663}) - 0.638 \times A_{645}]}{1000 \times W} \times V$$

Estimation of total carbohydrate content in plant extract

Leaves of *Cicer arietinum* (Chick pea) were dried at 80^0C for 48 h and then dried leaves (0.1g) were powdered and added to 3 ml of 80 % ethyl alcohol. Content was boiled in and then centrifuged for 15 min at 1,000 rpm. Supernatant was taken in a test tube and final volume was made 6 ml with 80 % ethyl alcohol. To 1ml ethanolic leaf extract, 4 ml ice cold Anthrone reagent was added. Mixture was shaken properly and boiled in water bath for 10 min. After cooling, absorbance was recorded at 620 nm. Amount of total sugar was estimated using a standard dilution taking glucose in the range of 10-100 µg/ml (Dubois *et al.* 1956).

Estimation of total protein content in plant extract

One gram fresh leaves were transferred to a mortar and pestle after thorough washing. After adding 5 ml of 0.2 M Tris-Cl (pH-8), leaves were crushed gently for 20 min until a fine slurry was formed. Slurry was centrifuged at 10,000 rpm at 4^0C for 20 min. Supernatant was transferred to fresh tube and stored at 4^0C for further use. To estimate protein according to Bradford (1976), 20µl supernatant was added to 280 µl of extraction buffer to which 3 ml comassie brilliant blue (CBB) G-250 was added. Mixture was kept at 37^0C for 5 min and absorbance was read at 595 nm in a spectrophotometer against a reagent blank. Amount of protein was calculated using a standard curve prepared with different concentrations of bovine serum albumin (10-100 µg/ml).

Fourier transform infrared spectroscopy of treated and untreated malachite green

FTIR analysis was done in the mid-IR region of 400-4000 cm^{-1} with 16 scan speed. The control dye sample and the sample extracted after decolorization mixed with KBr the samples were then ground, desorbed at 60 ^{0}C for 24 h and pressed to obtained IR transparent pellets. The absorbance FT-IR spectra of the samples were recorded using an FT-IR spectrum 2000 Perkin-elmer spectrometer. The FT-IR was first calibrated for background signal scanning with a control sample of pure KBr and then the experimental sample was scanned. The FT-IR spectrum of the control was finally subtracted from spectra of the non-degraded and degraded dyes (Gomare *et al.* 2009).

RESULTS AND DISCUSSION

The effluent sample was collected from common effluent of SIDCUL industries of Haridwar site was situated between north latitude 29° 56’49.75”N and

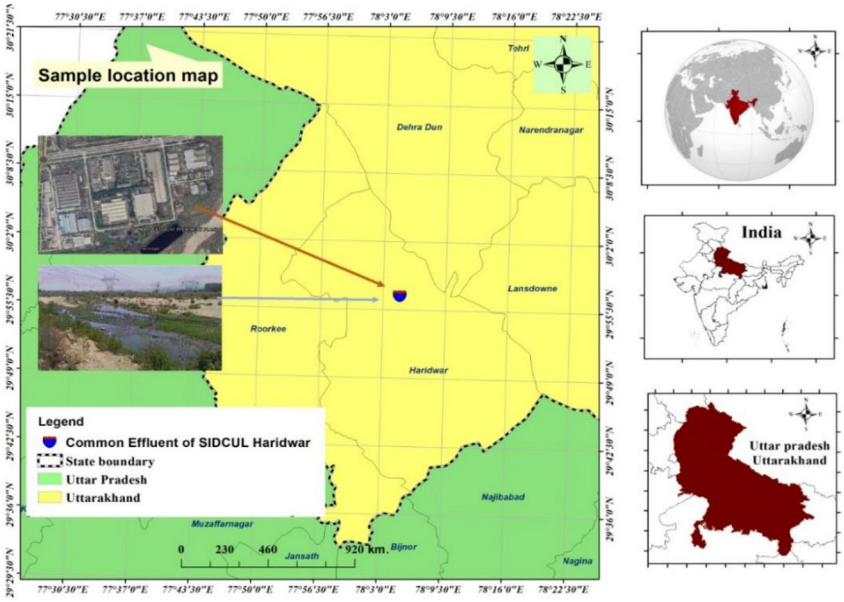

Fig. 1. Satellite view of sampling site.

east longitude 78° 4'46.23"E it is situated at a distance 11 km from the investigation laboratory (Fig.1).

A total of 15 morphologically different bacterial colonies were isolated from industrial effluent and screened out by repeated streaking on dye supplemented medium. From 14 bacterial strains eight bacterial strain were selected based on the primary visual identification and used for further studies (Table 1). Based on the dye degrading capacity eight selected bacterial strains were able to degrade malachite green used for further studies. After dye decolorization assay it was found that all the selected eight strains

Table 1. Ranking of dye decolorizer based on visual identification. Intense decolorization +++, Moderate decolorization ++, Slight decolorization + and - No. decolorization.

Sl.No.	Isolate	Ranking of decolorization
1.	4DD1	-
2	4DD2	+
3.	4DD3	++
4.	4DD4	-
5.	4DD5	-
6.	4DD6	-
7.	4DD7	-
8.	4DD8	++
9.	4DD9	-
10.	4DD10	++
11.	4DD11	++
12.	4DD12	-
13.	4DD13	++
14.	4DD14	++
15.	4DD15	+++

Table 2. Biochemical characterization of active decolorizing bacterial strain 4DD15. (+) = positive, (-) = negative, (A) =Absence.

Sl.No.	Biochemical test	4DD15
1.	Gram's reaction	+
2.	Shape	Bacilli
3.	MR	-
4.	VP	±
5.	Casien hydrolysis	+
6.	Starch	+
7.	Indole	-
8.	Urease	-
9.	Catalase	-
10.	Citrate	-
11.	H2S	-
12.	Gelatin	+
13.	Fermentation (Sucrose)	A
14.	Fermentation (Mannitol)	+
15.	Fermentation (Maltose)	+
16.	Fermentation (Lactose)	-
17.	Fermentation (Glucose)	+
18.	Fermentation (Glucose)	+

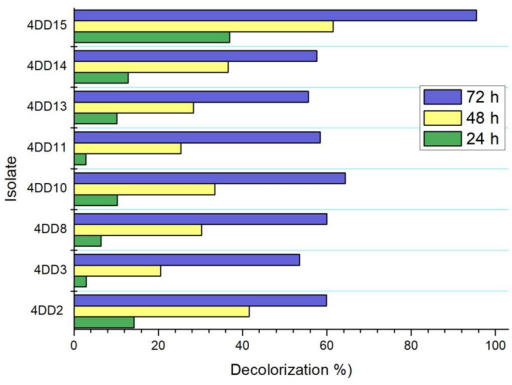

Fig. 2. Decolorization (%) of malachite green by various isolates..

were able to degrade malachite green. Among the eight bacterial strain 4DD15 (Fig. 2) exhibited maximum decolorization (95.52 %) of malachite green in 72 h. After the dye decolorization assay selected potent decolorizing strain 4DD15 was identified by gram staining and it indicated that 4DD15 was Gm + ve long rod shaped organism and the colonies of the organism grown on a nutrient agar the growth were white and moderate. Morphologically, cultural and biochemical characteristics of screened bacterial isolates are shown in Tables 2, 3. According to The Bergey's manual of determinative bacteriology and considering the physiological and biochemical tests performed, the strain was identified as *Bacillus* sp. strain 4DD15.

Table 3. Morphological characterization of active decolorizing bacterial strain 4DD15.

Sl.No.	Morphological	4DD15
1.	Size	Large
2.	Shape	Sphere
3.	Elevation	Slightly
4.	Margin	Undulate
5.	Opacity	Opaque
6.	Texture	Smooth

Identification was done by 16S rRNA analysis using universal primers. On the bases of NCBI GenBank database the strain has 98% identification similarities with the 16S rRNA gene sequence of the *Bacillus subtilis*. The presence of *Bacillus* sp. in dye containing effluent is a regular finding and many authors reported the role of these bacterial genera for the different degradation studies Karim *et al.* (2018). Thus, the selected bacterial isolate has been identified as *Bacillus subtilis*. Shakeel *et al*. (2020) reported similar observations for decolorization of a triphenylmethane dye by *Pseudomonas otitidis.* The phylogenetic relationship between the *Bacillus subtilis* strain 4DD15 and its closest related strain was constructed (Figs. 3,4).

Strain 4DD15 was further selected for optimization of cultural conditions. Decolorization of textile effluent at various temperatures suggests that a maximum decolorization 94.74% was achieved at 37°C within 72h (Fig.5a). For the determination of

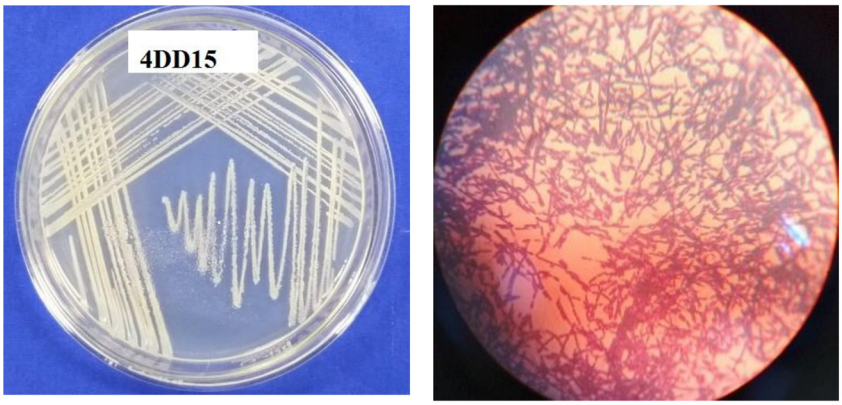

Fig. 3. Macroscopic and microscopic view of bacteria 4DD15 (*Bacillus subtilis*).

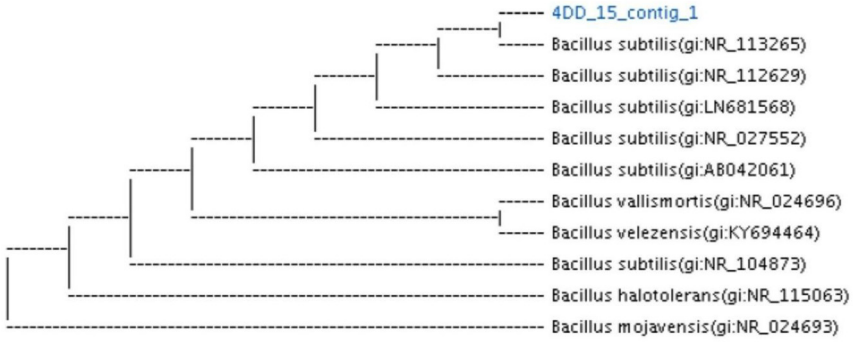

Fig. 4. Phylogenetic relationship between the *Bacillus subtilis* strain and its closest related strain.

optimal pH, experiments were carried out at different pH ranging from 5 to 9. The bacteria were able to grow and decolorize the dyes in all pH range, but the decolorization percentage was found to be high of 95.52% at the pH of 7 and decreases beyond and ahead of pH 7 (Fig.5b). Various carbon sources were used to replace original carbon source in culture medium to determine their role in malachite green decolorization. The results obtained showed that, sucrose brought the highest decolorization percentage of 79.03% followed by fructose with 74.93% of decolorization when compared to other carbon sources. Hence, sucrose was found to be the best carbon source for malachite green decolorization using *Bacillus subtilis.* (Fig. 5c). Various nitrogen sources were used in culture medium to determine their role in malachite green decolorization. Nigam *et al*. (1996) have reported maximum decolorization of dyes in the presence of yeast extract. Four different N_2 sources viz., yeast extract, beef extract, peptone and ammonium sulfate were tested in the present decolorization study the results obtained showed that, peptone 91.22 % brought the highest decolorization percentage of 79.03 % followed by beef extract with 76.31 % of decolorization when compared to other N_2 sources. Hence, peptone was found to be the best N_2 source for malachite green decolorization using *Bacillus subtilis* (Fig.5d).

To examine the effects of aeration on the level of efficiency of dyes decolorization two conditions, shaking and static were set. The levels of decolorization of malachite green were much higher under the shaking condition compared to the static one in all the five periods of incubation. The level of decolorization of malachite green reached its peak at 92.88 % after 120h of incubation under the shaking condition and 43.63% under static condition (Fig.5e).

The results of phytotoxicity studies using dye and its degraded products On *Cicer arietinum* are tabulated in Table 4 and effect on germination percentage and seedling growth in cm showed in (Figs. 6 a,b,c). Germination percentage was less with untreated dye as compared to bacterial treated dyes. The germination of the *Cicer arietinum* seeds in the presence of pure dye, degraded dye and water control showed 70%, 90% and 100% respectively. Inhibition of germination of 30% indicating that pure dye was toxic to the plant. The length of plumule and radicle of germinating seeds was taken after seven days and recorded. The length of plumule and radical were significantly affected by malachite green than its degraded metabolites. The control seeds, irrigated with water showed better growth and the average length of plumule and radicle 6.1 cm and 2.5 respectively. The seeds irrigated with dyes showed retardation in growth and the seeds irrigated with dye showed the maximum growth of plumule and radicle 2.22 cm , .52 cm respectively. Degraded dye showed 5.81 plumule and 2.1 cm radicle better results than untreated dye. The shoot and root length were drastically affected by dye.

Chlorophyll content decreased in the *Cicer arietinum* plants leave those irrigated with dye showed the maximum amount of chlorophyll content 0.85+0.09 in water irrigated plants, 0.596 ± 0.02 in

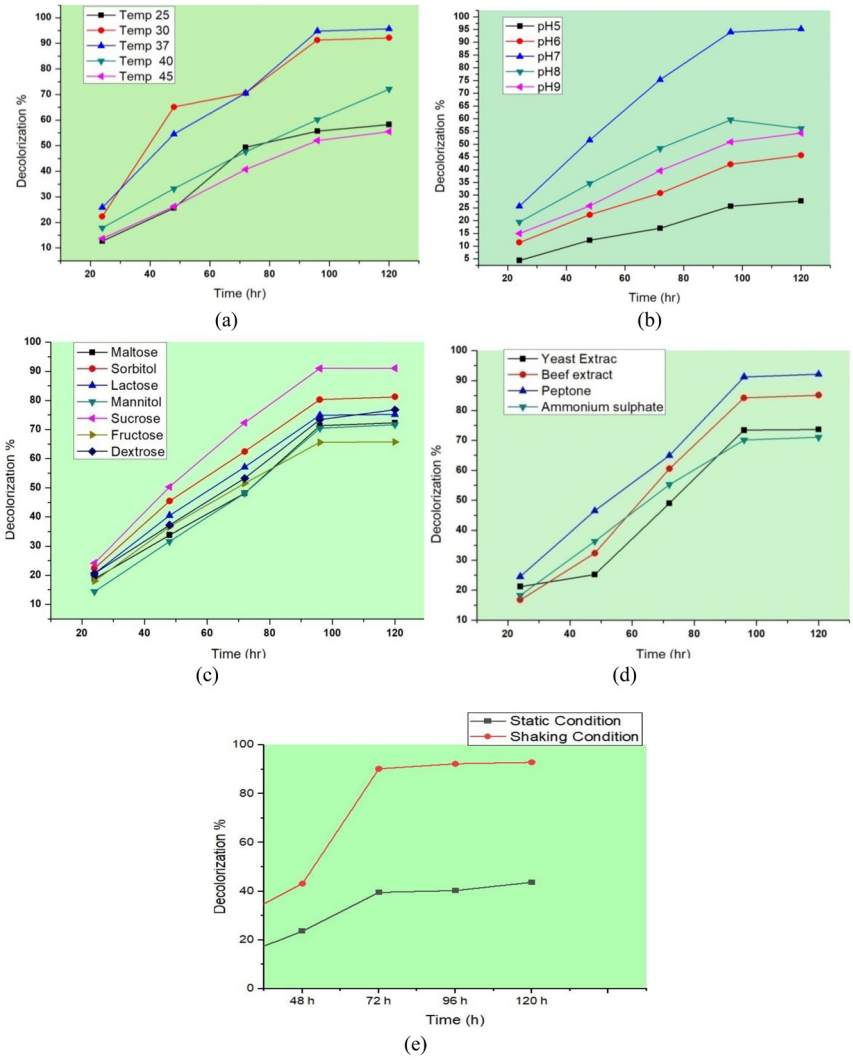

Fig. 5. Effect of various parameters on the decolorization of malachite green by *Bacillus subtilis* (a) effect of different temperature (b) effect of different pH (c) effect of different carbon sources (d) effect of different nitrogen sources (e) effect of static and shaking condition.

degraded dye metabolites irrigated plants and lowest chlorophyll content 0.41±0.02 estimated in the plants irrigated with dye. It was observed that the total sugar, carotenoid and protein significantly affected by the malachite green. Different parameter total sugar, carotenoid and protein of *Cicer arietinum* plant showed 12.48±0.21, 7.41±0.34 and 0.016±0.0001 respectively in the plant irrigated with dye and 18.68±0.29, 14.53±0.32 and 0.086±0.051 respectively in the plant irrigated with degraded dye with *Bacillus subtilis.* The plant irrigated with dye showed retardation in different parameter total sugar, carotenoid and protein. Awad *et al.* (2019), Unuofin *et al.* (2019), Sharma *et al.* 2020) observed that highest germination of *T. aestivum* seeds were in the presence of decolorized malachite green (Figs. 7 a,b,c).

From the FTIR analysis it was concluded that decolorization of malachite green by means of degradation which causes changes in the molecular

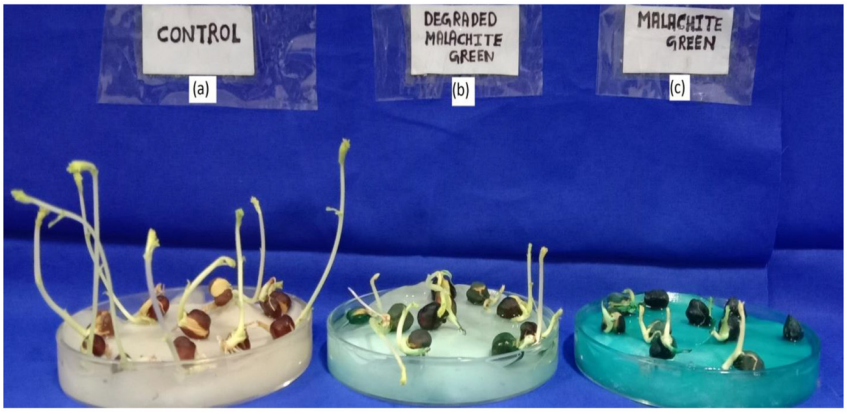

Fig. 6. Germination of seeds of *Cicer arietinum* under *in vitro* conditions irrigated with a) Water control b) Malachite green dye degraded by *Bacillus subtilis* c) Malachite green dye respectively from left to right.

Table 4. Phytotoxicity study of dye and its degraded products on the different parameter of *Cicer arietinum* seeds. Value are the mean + SEm data were analyzed by one-way analysis of variance (ANOVA) with Tukey-kramer multiple comparisons test. Readings were considered significant when p was ≤ 0.05.

Parameter	Distilled water	Malachite green	Degraded malachite green
Total chlorophyll (mg g^{-1})	0.83±0.008	0.48±0.003	0.62±0.006
Carotenoid (mg g^{-1})	0.03±0.0008	0.019±0.0005	0.023±0.0009
Protein (mg g^{-1})	22.38±0.36	8.52±0.29	16.14±0.15
Total sugar (mg g^{-1})	33.08±0.09	12.48±0.21	21.4±0.26
Germination %	100	70	90
Plumule	7.04±0.157	2.22±0.292	5.81±0.184
Radicle	2.5±0.255	1.52±0.220	2.1±0.063

orientation of the pure dye molecule, it results in the formation of the different fragments indicated by the formation of new peaks in FTIR spectra. It was concluded that the action of the selected bacterium species *Bacillus subtilis* on dye molecules malachite green and resulted in to the formation of dye products. FTIR is one of the most widely used techniques to determine the product formed from the original chemical compound. Many researchers used FTIR for dye decolorization studies.

A presents fourier transform infra-red spectrum

Fig. 7. Germination and growth of *Cicer arietinum* under *in vivo* conditions irrigated with a) Water control b) Malachite green dye degraded by *Bacillus subtilis* c) Malachite green dye respectively from left to right.

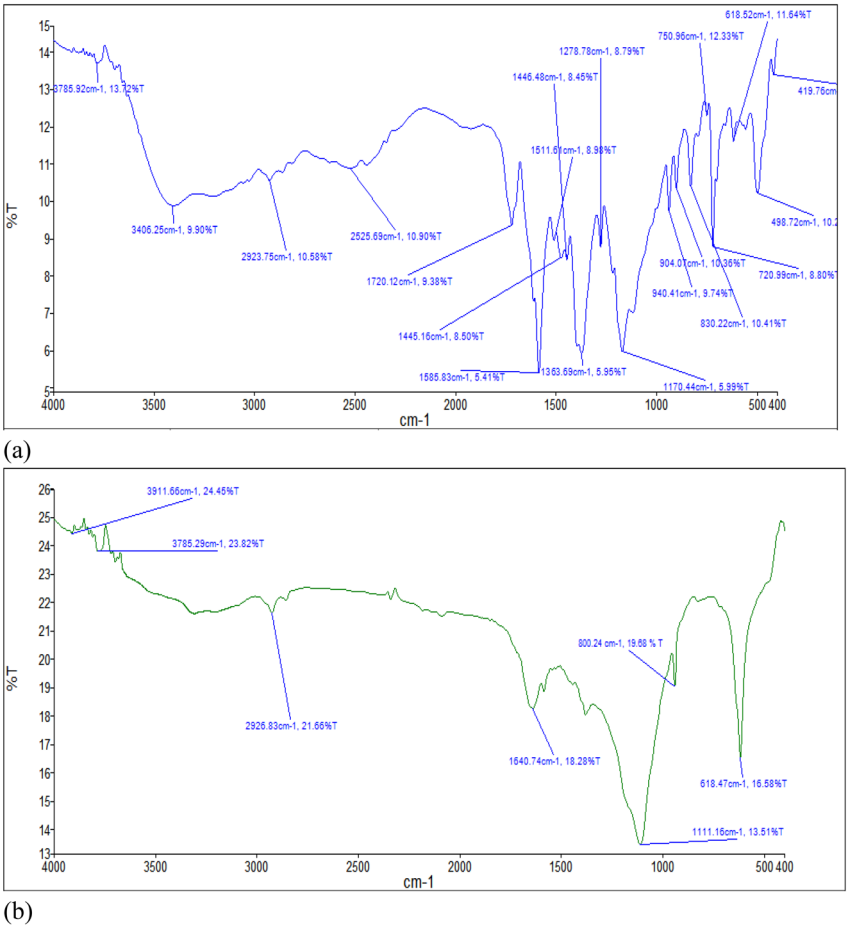

Fig. 8. (a, b). FTIR spectrum of malachite green (control) degraded malachite green dye extracted after 72 h of degradation by *Bacillus subtilis.*

of control malachite green displaying various peaks in the finger print region (1500 to 500 cm^{-1}) for para-disubstituted and meta-disubstituted benzene rings as well as a peak at 1585.83 cm^{-1} for benzene for the C=C stretching for benzene ring. A peak at 940.41cm^{-1} for O-H bend of carboxylic acid and peak at 1170.44 cm^{-1} for C=N stretch and peak at 2923.7 cm^{-1} for C-H stretching of asymmetric CH_3 group gives the indication of malachite green. The samples for FTIR were obtained after 72h incubation of malachite green with *Bacillus subtilis*. The results of FTIR spectra of the sample obtained (extracted) after decolorization with *Bacillus subtilis* showed absence of peak between (850-600 cm^{-1}) indicates breakdown of aromatic ring of malachite green and crystal violet bond. The absence of peak at 720.99 cm^{-1} indicates loss of aromaticity or benzene ring. The vibrations in the finger print region (1500 to 500 cm^{-1}) of the FTIR spectrum of control (Fig.8a) and extracted metabolite after 72h degradation and by *Bacillus subtilis* (Fig.8b). It was reported that biotransformation occurs due to formation of oximes and imines or nitroso compound might be rearranged into oximes. FTIR analysis confirms biotransformation of dye into the nitroso compound, oximes and imines. Degradation of malachite green by *Kocuria rosea* MTCC 1532 was confirmed by FTIR analysis (Shakeel *et al.* 2020, Amiri *et al.* 2020) concluded from FTIR analysis that *S. cerevisiae* MTCC 463 effectively decolorizes methyl red in to the fragments

by biodegradation Shariffah-Muzaimah *et al.* (2018) confirmed the degradation of reactive yellow 84A by *Exiguobacterium* sp. by using the FTIR analysis.

CONCLUSION

From the finding of performed research we concluded *Bacillus subtilis* is potent bacteria for dye decolorization and this strain can be used successfully in future for decolorization. *Bacillus subtilis* showed maximum decolorization at pH 7, temperature 37°C, in the presence of sucrose as a carbon source and peptone as a nitrogen source in shaking condition. Phytotoxicity analysis showed higher seed germination percentage, seedling growth and other growth parameter in the presence degraded dye. We conclude that degraded dye was nontoxic for *Cicer arietinum* (Chickpea). FITR analysis on MG degraded metabolites depicted the formation of aromatic amines, carboxylic acids, alkanes, alkenes, which shows the formation of non-toxic metabolites during degradation.

ACKNOWLEDGEMENT

Authors are thankful to the Department of Microbiology, Girls campus, Gurukul Kangri Vishwavidhyalaya, Haridwar providing all the necessary facilities required to carry out the research work.The authers are also thankful to Biokart India Pvt Ltd providing molecular facilities and IIT Roorkee for providing FTIR analysis facilities.

REFERENCES

Ajaz M, Shakeel S, Rehman A (2020) Microbial use for azo dye degradation—a strategy for dye bioremediation. *Int Microbiol* 23 (2): 149-159.

Ali SS, Al-Tohamy R, Xie R, El-Sheekh MM, Sun J (2020) Construction of a new lipase-and xylanase-producing oleaginous yeast consortium capable of reactive azo dye degradation and detoxification. *Biores Technol* 313: 123-131.

Al-Tohamy R, Sun J, Fareed MF, Kenawy ER, Ali SS (2020) Ecofriendly biodegradation of reactive black 5 by newly isolated *Sterigmatomyces halophilus* SSA1575, valued for textile azo.

Amin AS, Kassem MA (2012) Chromium speciation in environmental samples using a solid phase spectrophotometric method. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy* 96: 541-547.

Amiri M, Dashtian K, Ghaedi M, Mosleh S (2020) A dual surface inorganic molecularly imprinted Bi 2 WO 6-CuO/Ag 2 O heterostructure with enhanced activity-selectivity towards the photocatalytic degradation of target contaminants. *Photochem Photobiol Sci* 19(7): 943-955.

An Q, Cheng JR, Wang YT, Zhu MJ (2020) Performance and energy recovery of single and two stage biogas production from paper sludge: *Clostridium thermocellum* augmentation and microbial community analysis. *Renewable Energy* 148: 214-222.

Atallah E, Kwapinski W, Ahmad MN, Leahy JJ, Ala'a H, Zeaiter J (2019) Hydrothermal carbonization of olive mill wastewater: Liquid phase product analysis. *J Environ Chem Engg* 7 (1): 102833.

Awad AM, Shaikh SMR, Jalab R, Gulied MH, Nasser MS, Benamor A, Adham S (2019) Adsorption of organic pollutants by natural and modified clays: A comprehensive review. *Separ Purif Technol* 228 : 115719. https://doi.org/10.1016/j.seppur.2019.115719.

Barathi S, Arulselvi PI (2015) Isolation and characterization of textile dye degrading native bacterial strains from textile effluent contaminated sites. *Int J Multidisci Res De*v 2(4): 155-160.

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72(1-2): 248.

Buchanan RE, Gibbons NE (1974) Bergey's Manual of Deter minative Bacteriology, 8th ed. The Williams and Wilkins Company, Baltimore.

Dubois M, Gilles KA, Hamilton JK, Rebers PT, Smith F (1956) Colorimetric method for determination of sugars and related substances. Anal Chem 28(3): 350-356.

Gomare SS, Parshetti GK, Govindwar SP (2009) Biodegradation of malachite green by *Brevibacillus laterosporus* MTCC 2298. *Water Environ Res* 81(11): 2329-2336.

Karim ME, Dhar K, Hossain MT (2018) Decolorization of textile reactive dyes by bacterial monoculture and consortium screened from textile dyeing effluent. *J Genet Engg Biotechnol* 16 (2): 375-380.

Kirk JTO, Allen RL (1965) Dependence of chloroplast pigments synthesis on protein synthetic effects on actilione. Hazardous Materials Biochem. Biophysics Res J Canada 27: 523-530. Method," Journal of Hazardous Materials, Vol. 143, . 214–219, 2007.

Nigam P, Banat IM, Singh D, Marchant R (1996) Microbial process for the decolorization of textile effluent containing azo, diazo and reactive dyes. *Proc Biochem* 31: 435 - 442.

Pourrahim S, Salem A, Salem S, Tavangar R (2020) Application of solid waste of ductile cast iron industry for treatment of wastewater contaminated by reactive blue dye via appropriate nano-porous magnesium oxide. *Environ Pol* 256: 113454.

Qi W, Wang J, Quan X, Chen S, Yu H (2020) Catalytic ozonation by manganese, iron and cerium oxides on γ-Al2O3 pellets for the degradation of organic pollutants in continuous fixed-bed reactor. *Ozone: Sci Engg* 42(2): 136-145.

Riegas-Villalobos A, Martínez-Morales F, Tinoco-Valencia R, Serrano-Carreón L, Bertrand B, Trejo-Hernández MR (2020) Efficient removal of azo-dye Orange II by fungal biomass absorption and laccase enzymatic treatment. *3 Biotech* 10 (4): 1-10.

Shah MP, Patel KA, Nair SS, Darji AM (2013) Optimization of environmental parameters on microbial degradation of reactive black dye. J Bioremed Biodegrad 4 (3): In press.

Shakeel M, Imran M, Ashraf M, Ullah N (2020) Biodegradation by co-inoculated bacteria and fungi alleviates adverse effects of red S3B on growth and nitrogen uptake of wheat. Clean–Soil, Air, Water 48 (3): 1900305.

Shariffah-Muzaimah SA, Idris AS, Madihah AZ, Dzolkhifli O, Kamaruzzaman S, Maizatul-Suriza M (2018) Characterization of Streptomyces spp. isolated from the rhizosphere of oil palm and evaluation of their ability to suppress basal stem rot disease in oil palm seedlings when applied as powder formulations in a glasshouse trial. World J Microbiol Biotechnol 34(1): 1-14.

Sharma A, Gupta G, Ahmad T, Kaur B, Hakeem KR (2020) Tailoring cellular metabolism in lactic acid bacteria through metabolic engineering. J Microbiol Methods 170: 105862.

Telke AA, Joshi SM, Jadhav SU, Tamboli DP, Govindwar SP (2010) Decolorization and detoxification of congo red and textile industry effluent by an isolated bacterium Pseudomonas sp. SU-EBT. Biodegradation 21: 283-296.

Unuofin JO, Moubasher HA, Okoh AI, Nwodo UU (2019) Production of polyextremotolerant laccase by Achromobacter xylosoxidans HWN16 and Citrobacter freundii LLJ16. Biotechnol Rep 22: e00337.

Upadhyay GK, Rajput JK, Pathak TK, Pal PK, Purohit LP (2020) Tailoring and optimization of hybrid ZnO: TiO2: CdO nanomaterials for advance oxidation process under visible light. Appl Surface Sci 509: 145326.

Verma A, Dhiman K, Shirkot P (2017) Biodegradation of malachite green by extracellular bacterial laccase and its phytotoxicity studies. Int J Pure Appl Biosci 5(2): 913-922.

Wellburn AR (1994) The spectral determination of chlorophyll a and b, as well as total carotenoids, using various solvents with spectrophotometers of different resolution. J Pl Physiol 144: 307-313.