Scientific Paper

# Biodegradation of Malachite Green by *Kocuria rosea* MTCC 1532

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## Abstract

Malachite green (50 mg/L) was completely decolorized under static anoxic condition within 5 h by bacteria *Kocuria rosea* MTCC 1532; however decolorization was not observed at shaking condition. *K. rosea* have also shown decolorization of azo, triphenylmethane and industrial dyes (cotton blue, methyl orange, reactive blue 25, direct blue-6, reactive yellow 81, and red HE4B). Semi-synthetic media containing molasses, urea and sucrose have shown 100, 91, 81% decolorization respectively. Induction in the activities of malachite green reductase and DCIP reductase was observed during MG decolorization suggesting their involvement in the decolorization process. UV-Visible absorption spectrum, HPLC and FTIR analysis showed degradation of MG. Toxicity study revealed the degradation of MG into non-toxic products by *K. rosea.*

**Keywords:** decolorization, *K. rosea*, malachite green reductase, HPLC, toxicity.

## 1. Introduction

A major class of synthetic dyes includes the azo, anthroquinone and triphenylmethane dyes. Dyes are difficult to degrade biologically, so that degradation of dyes has received considerable attention. About 10-15% of all dyes are directly lost to wastewater in the dyeing process.[1-2] Thus, the wastewater must be treated before releasing into the natural environment. For biological treatment of the wastewater containing dyes, the microbial decolorization and degradation of dyes has been of considerable interest.[1] Aerobic degradation of triphenylmethane dyes has been demonstrated repeatedly; however these dyes resist degradation in activated sludge systems.[3] MG is used extensively for dyeing silk, wool, jute, leather, ceramics, cotton and used to treat fungal and protozoal infection.[4] The members of triphenylmethane family are animal carcinogens. The Food and Drug Administration nominated MG as a priority chemical for carcinogenicity testing by the National Toxicology Program 1993.[4] MG and its reduced form, leucomalachite green, may persist in edible fish tissues for extended periods of time.[5] Therefore, there are both environmental and human health concerns about bioaccumulation of MG and leucomalachite green in terrestrial and aquatic ecosystems. The initial reduction of MG using an intestinal bacterium[6] has been already reported; recently our laboratory has shown 85% MG decolorized within 7 h by yeast.[2] Among other microorganisms, white rot fungi proved their ability to efficiently degrade malachite green.[7-8] Anaerobic dye reduction is mainly a biological process, either a direct enzymatically catalyzed reaction involving non-specific enzymes or a reaction with enzymatically reduced electron carriers.

The aim of present study was to investigate the fastest decolorization of MG by *K. rosea* and also to study the possible enzymes involved in decolorization as well as to access the toxicity of MG degradation product. Various conditions for decolorization have been optimized.

## 2. Experimental

### 2.1. Dyes and chemicals

Malachite green (MG) was obtained from S d Fine Chemicals Limited (Biosar, India) and ABTS (2, 2-Azinobis (3-ethylbenzothiazolin-6-sulfonic acid) from Sigma Chemical Company (St. Louis, MO, USA). Cotton blue, methyl orange, methyl violet, crystal violet, methyl orange, amido black, reactive blue 25, direct blue 6, reactive yellow 81, red HE4B and reactive green 19 A were purchased from a local market and used for this study. Tartaric acid was obtained from BDH Chemicals (Mumbai, India). n-propanol, catechol and other fine chemicals were from SRL Chemicals, India. All chemicals used were of the highest purity available and of an analytical grade.

### 2.2. Organism and culture condition

The strain *Kocuria rosea* MTCC 1532 used in this study was obtained from Institute of Microbial

Technology, Chandigarh, India. It was maintained on nutrient agar slants at 4 °C. The pure culture of *K. rosea* was grown in 250 mL Erlenmeyer flask, containing 100 mL nutrient broth (g/L: beef extract 1, yeast extract 2, peptone 5 and NaCl 5) at 30 °C for 24 h at static anoxic condition. To study the effect of carbon and nitrogen sources on decolorization of malachite green, semi-synthetic medium having following composition was used. (g/L): MG 0.050, $(NH_4)_2SO_4$ 0.28, $NH_4Cl$ 0.23, $KH_2PO_4$ 0.067, $MgSO_4.7H_2O$ 0.04, $CaCl_2.2H_2O$ 0.022, $FeCl_3$. $6H_2O$ 0.005, yeast extract 0.2, NaCl 0.15, $NaHCO_3$ 1.0, and 1 mL/L of a trace element solution containing (g/L) $ZnSO_4.7H_2O$ 0.01, $MnCl_2.4H_2O$ 0.1, $CuSO_4.5H_2O$ 0.392, $CoCl_2.6H_2O$ 0.248, $NaB_4O_7.10H_2O$ 0.177, and $NiCl_2.6H_2O$ 0.02 with different carbon and nitrogen sources such as, glucose (1 and 3%), malt extract (1%), sucrose (1%), molasses (0.5%), peptone (1%) and urea (1%).

**2.3. Decolorization experiments**

All decolorization experiments were performed in three sets. A loopful of microbial culture was inoculated in 250 mL Erlenmeyer flask containing 100 mL nutrient broth and incubated at 30 °C for 24 h. After 24 h of incubation, all dyes were added at concentration of 50 mg/L and 3 mL of the culture media was withdrawn at different time intervals. Aliquot was centrifuged at 5000 rpm for 15 minutes to separate the bacterial cell mass, clear supernatant was used to measure the decolorization at the absorbance maxima of the respective dyes. Abiotic controls (without microorganism) were always included.

*K. rosea* cells grown for 24 h in nutrient broth were used to monitor decolorization of MG at static and shaking (120 rpm, 30 °C) condition. Decolorization at various increasing concentrations of MG (10-100 mg/L) and industrial dyes (dye concentration 50 mg/L) were tested at 30 °C in nutrient broth at static condition. Studies on effect of various carbon and nitrogen sources on decolorization of MG (50 mg/L) were carried out in semi-synthetic medium at static condition (30 °C). 10% inoculum of optical density 1.0 (620 nm) grown in nutrient broth for 24 h was used for inoculation of semi-synthetic medium.

The percentage decolorization was calculated as follows

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

**2.4. Preparation of cell free extract**

*K. rosea* cells were grown in nutrient broth at 30 °C for 24 h. Cells were centrifuged at 10,000 rpm for 20 minutes. These cells (75 mg/mL) were suspended in potassium phosphate buffer (50 mM, pH 7.4) for sonication (sonics-vibracell ultrasonic processor), keeping sonifier output at 40 amp, giving 7 strokes, each of one second with one minute interval. The temperature was maintained below 4 °C. This extract was used as source of enzyme. Similar procedure was followed to cells obtained after decolorization (5 h).

**2.5. Enzyme analysis**

Activities of laccase, tyrosinase and lignin peroxidase were assayed spectrophotometrically in cell free extract as reported earlier.[1] Laccase activity was determined by measuring oxidation of ABTS at 420 nm in a reaction mixture of 2 mL containing 10% ABTS in 0.1 M acetate buffer (pH 4.9). Tyrosinase activity was determined in a reaction mixture of 2 mL, containing 0.01% catechol in 0.1 M phosphate buffer (pH 7.4) by measuring liberated catechol quinone at 410 nm. Lignin peroxidase activity was determined by monitoring the formation of propanaldehyde at 300 nm in a reaction mixture of 2.5 mL containing 100 mM n-propanol, 250 mM tartaric acid, 10 mM $H_2O_2$. All enzyme assays were carried out at room temperature. Reference blanks contained all components except the enzyme. One unit of enzyme activity was defined as a change in absorbance unit per minute per mL of enzyme.

NADPH-DCIP (Dichorophenol indophenol) reductase activity was determined by procedure reported earlier.[9] In the Malachite green reductase (MG reductase) activity [2] the assay mixture contained 323 μM malachite green, 50 μM NADH in 50 mM potassium phosphate buffers (pH 7.4) and 0.1 mL of enzyme solution in a total volume of 5.0 mL. The decrease in color intensity of MG was observed at 620 nm. The MG reduction was calculated using the extinction coefficient (ε) of $8.4 \times 10^{-3}$ $mM^{-1}$ $cm^{-1}$.

**2.6. Biodegradation analysis**

TLC, UV-Visible Spectroscopy, HPLC and FTIR analysis.

After complete decolorization (5 h incubation), the decolorized medium was centrifuged at 10,000 rpm for 20 minutes and supernatant obtained was used to extract metabolites with equal volume of ethyl acetate. The extracts were dried over anhydrous $Na_2SO_4$ and evaporated to dryness in rotary evaporator. The crystals obtained were dissolved in small volume of methanol and used for analysis. Metabolite formation was examined by thin layer chromatography (TLC) using silica gel activated in the chloroform. The solvent system used was n-propanol: ethyl acetate: acetic acid: distilled water (6:1:1:2 v/v). The visualization of separated products was done in iodine chamber. UV-Visible spectral analysis was carried out using Hitachi UV-Visible spectrophotometer (UV 2800) and changes in the absorption spectrum of the decolorized

medium (400-800 nm) were recorded and compared with the control. HPLC (High performance liquid chromatography) analysis was carried out at 265 nm on waters model no. 2690 equipped with dual λ UV-Visible detector and $C_{18}$ column (symmetry, 4.6 × 250 mm). The mobile phase was the gradient of methanol and water with 40% methanol in water for two minutes, with flow rate of 0.5 mL/minute followed by 10 minutes linear gradient to 95% methanol and this gradient was held for additional 8 minutes with the same flow rate. The Fourier Transform Infrared Spectroscopy (FTIR) analysis of extracted metabolites was done on Perkin Elmer, Spectrum one instrument and compared with control dye in the mid IR region of 400-4000 $cm^{-1}$ with 16 scan speed. The samples were mixed with spectroscopically pure KBr in the ratio of 5:95, pellets were fixed in sample holder, and the analyses were carried out.

### 2.7. Toxicity study

The ethyl acetate extracted products of MG degradation were dried and dissolved in 5 ml sterile distilled water to form a final concentration of 100 ppm for phytotoxicity studies. The phytotoxicity study was carried out (at room temp) in relation to *Triticum aestivum* and *Phaseolus mungo* (10 seeds of each) by watering separately 5 mL sample of control MG and its degradation product (100 ppm) per day. Control set was carried out using distilled water at the same time. Germination (%) and length of plumule (shoot) and radicle (root) was recorded after 7 days. Microbial toxicity of control MG and its degradation product (ethyl acetate extracted and dried, final concentration of 500 and 1000 ppm in 5 ml) was also carried out in relation to *K. rosea, Pseudomonas aeruginosa* and *Azotobacter vinelandii* and zone of inhibition (diameter in cm) was recorded after 24 h of incubation at 30 °C.

### 2.8. Statistical analyses

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.

## 3. Results and Discussion

### 3.1. Decolorization at static and shaking condition

*K. rosea* showed 100% decolorization of MG (50 mg/L) within 5 h at static anoxic condition whereas there was no decolorization at shaking condition (data not shown). The reason for no decolorization at shaking condition could be competition of oxygen and dye for the reduced electron carriers under aerobic condition.[1] There was no abiotic loss of MG within 5 h of incubation. It has been shown that the cultures of *C. elegans* transformed apparently 85% of MG (81 mM) in culture flasks within 24 h. A concentration of 108 mM MG inhibited fungal growth, [7] and biotransformation did not occur. This could be first report on 100% decolorization of MG within 5 h at 50 mg/L concentration by *K. rosea*. To confirm whether this decolorization was due to bacterial action or change in pH, the change in pH was recorded which was in the range of 6.8-6.9. UV-Visible spectra of MG did not show any change at this pH range (data not shown).

### 3.2. Effect of initial dye concentration on decolorization

The decolorization of MG was studied at various increasing concentration of dye i.e. from 10, 30, 50, 70 and 100 mg/L. We found that the rate of decolorization was decreased with increasing concentration of dye (data not shown). Similar observation was also reported during decolorization of reactive violet 5 by newly isolated bacterial consortium.[10] 100% decolorization of MG was observed at 10, 30 and 50 mg/L concentrations within 2, 3 and 5 h respectively. The rate of decolorization was decreased beyond the 50 mg/L dye concentration, indicating reduction in decolorization with increase in dye concentration. Only 13 and 6% decolorization was observed at 70 and 100 mg/L dye concentration respectively. These results indicate toxicity of MG at higher dye concentration.

### 3.3. Effect of carbon and nitrogen source on decolorization

In semi-synthetic medium, only 7% decolorization of MG was observed in 60 h. In an attempt to enhance decolorization performance with extra supplements of carbon and nitrogen source in semi-synthetic medium, we found 100% decolorization with molasses (0.5%), whereas 91, 81, 40 and 31% decolorization was observed with urea (1%), sucrose (1%), peptone (1%) and glucose (3%), respectively in 12 h. No decolorization was observed with glucose (1%) and malt extracts (1%) (data not shown). Further 100% decolorization was observed after 24 h with urea and sucrose. Presence of molasses, sucrose and urea in semi-synthetic medium showed maximum decolorization of MG compared to peptone and glucose.

### 3.4. Decolorization of industrial dyes

Decolorization of some azo (methyl orange, amido black, congo red) triphenylmethane (cotton blue, methyl violet, crystal violet) and textile dyes such as monochloro-triazine dyes (reactive blue 25, direct blue-6, reactive yellow 81, reactive red 141, reactive green 19 A) were tested with *K. rosea* (dye conc. 50 mg/L each). Complete decolorization of cotton

blue required 7 days. No decolorization was observed in case of methyl violet and crystal violet. Out of three azo dyes only methyl orange was decolorized within 3 days. When monochloro triazine dyes were tested for decolorization, color change was observed from blue to green with reactive blue 25 in 24 h and no further decolorization. Direct blue-6 and reactive red 141 were completely decolorized within 7 days, whereas reactive yellow 81 took 13 days (Figure 1). No decolorization was observed with reactive green 19 A. These results indicate *K. rosea* can be used for decolorization of some industrial dyes.

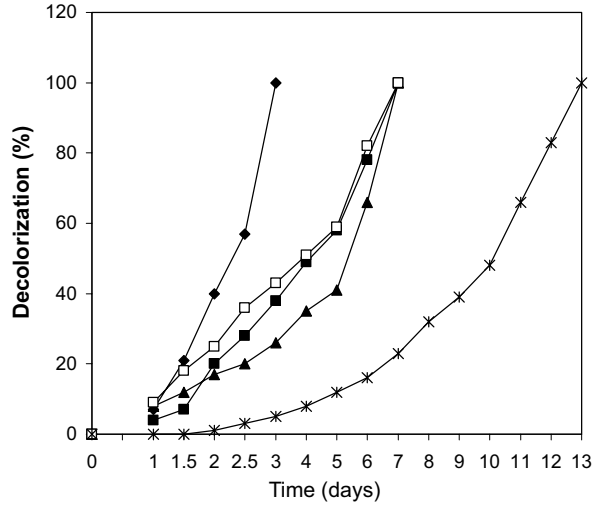


**Figure 1.** Decolorization of methyl orange (◆), reactive yellow 81 (∗), direct blue 6 (■), reactive red 141 (▲) and cotton blue (□) by *k. rosea*.

### 3.5. Enzyme Analysis

Studies on the biodegradation of triphenylmethane dyes have focused primarily on the decolorization of dyes via reduction reactions.[3, 11] MG reduction to leucomalachite green and further into its derivative have been reported earlier.[5, 12] In our study autoclaved cell free extract of *K. rosea* did not show decolorization of MG, which indicates enzymatic reaction. Cell free extract contains a protein that may be responsible for MG decolorization.[2] Cell free extract of *K. rosea* showed the presence of the enzymes responsible for dye degradation viz. DCIP reductase, MG reductase, laccase, tyrosinase and lignin peroxidase. No induction in laccase, lignin peroxidase and tyrosinase activity was observed during decolorization process (Table 1). The significant induction in DCIP reductase (12%) and MG reductase (73%) activities were observed during decolorization of MG by *K. rosea*. This indicates the involvement of reductase enzymes in MG degradation by *K. rosea*. Azoreductase activity in *Pseudomonas sp.* and *Klebsiella sp.* was inducible [13] and most reductases are involved in decolorization process. [14-15] Recently our laboratory have reported involvement of MG reductase in the decolorization of MG by *Saccharomyces cerevisiae* and activity was induced in the cells after decolorization of MG.[2]

**Table 1.** Enzyme activities in control (0 h) and Induced state (after 5 h decolorization).

| Enzyme assay | Control | Induced |
|---|---|---|
| DCIP Reductase[a] | 47.33 ± 1.33 | 53.23* ± 0.33 |
| MG reductase[b] | 0.15 ± 0.01 | 0.26* ± 0.03 |
| Laccase[c] | 0.006 ± 0.001 | ND |
| Tyrosinase[d] | 0.0058 ± 0.005 | ND |
| Lignin Peroxidase[e] | 0.0083 ± 0.0008 | 0.0058 ± 0.0008 |

ND, Not in detectable range,
[a] $\mu$g DCIP reduced/ minute/mg protein
[b] $\mu$g MG reduced/ minute/ mg protein,
[c,d,e] Enzyme activity - units/ mL/ min.
Values are mean of three experiments ± SEM, Significantly different from control cells at $^{*}P \leq 0.05$ by One Way ANOVA with Tukey-Kramer multiple comparisons test.

### 3.6. Analysis of degradation products

TLC of extracted metabolites confirmed the degradation of MG. The RF value of MG was noted as 0.61 where as extracted metabolites had shown two spots with the increased Rf values as 0.72 and 0.82 (data not shown). The shift in $\lambda_{max}$ value of MG (618 nm) to lower wavelength observed in decolorized medium confirms biodegradation of MG (Figure 2).

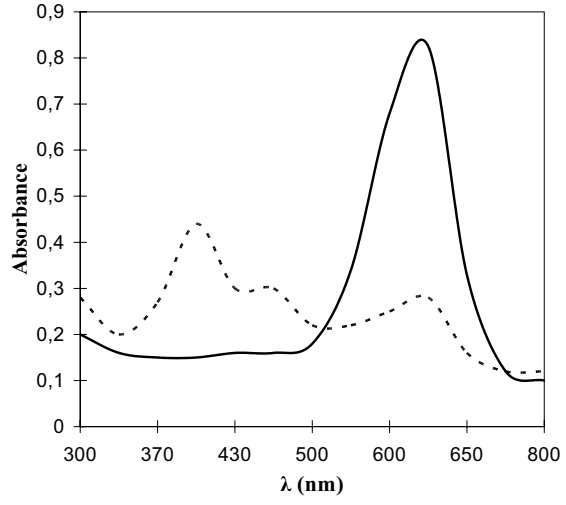


**Figure 2.** Spectrophotometrically analysis of Malachite green at 0 h (—) and after 5 h (----) decolorization by *K. rosea*.

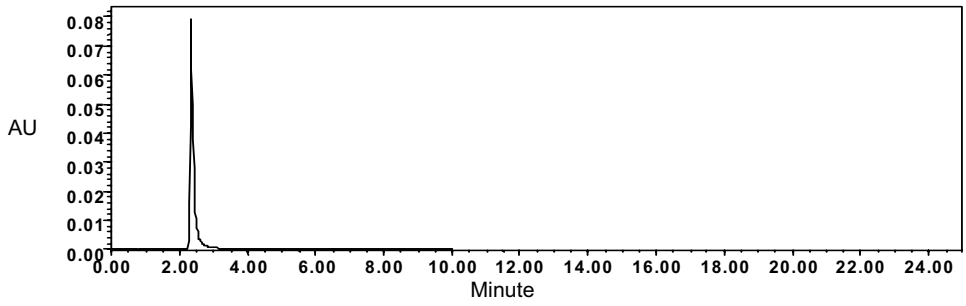


Figure 3 a.

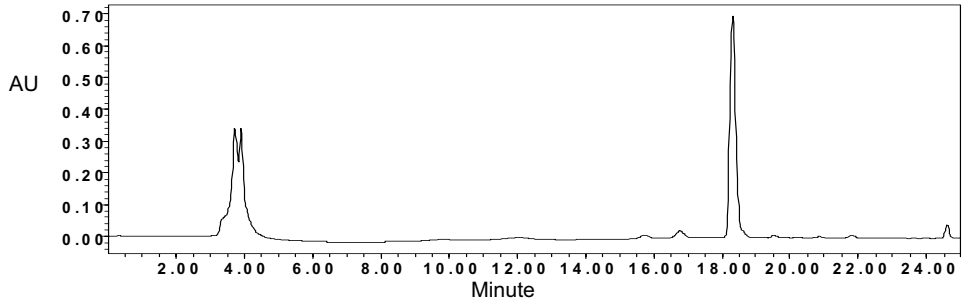


Figure 3 b.

**Figure 3.** HPLC elution profile of malachite green and its metabolites by *K. rosea*.
(a) Chromatogram of malachite green (0 h), (b) chromatogram of 5 h extracted metabolites.

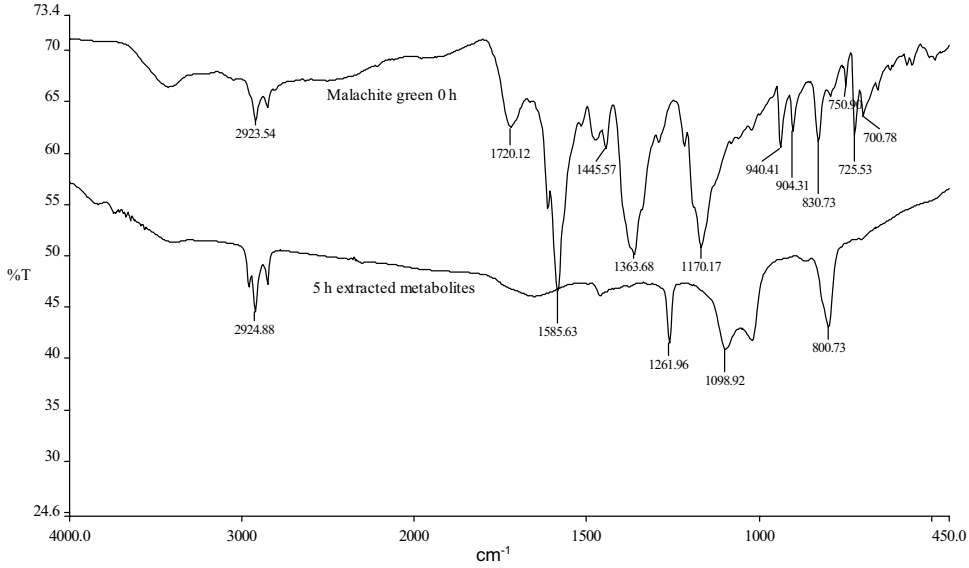


**Figure 4.** FTIR spectrum of malachite green dye 0 h (control) and metabolites extracted after 5 h of degradation by *K. rosea*.

This result suggests that some demethylated products might have formed during the degradation, since demethylated products shows absorption maxima at wavelength lower than that of MG.[7] HPLC chromatogram of extracted sample after complete decolorization (Figure 3a, b) also confirmed the degradation of MG into metabolites. Control MG showed retention time at 2.33 minutes and metabolites were observed at 3.73, 3.98 and 18.31 minutes. Formation of leucomalachite green and various demethylated derivatives during the degradation of MG was observed and only leuco derivatives were observed as a final product of biotransformation, after a prolonged incubation time (10 days) in *Cunninghamella elegans*.[7]

However, this strain showed appearance of the peaks at different retention time than leucomalachite green (3.86 minutes), which indicates that the products are other than leucomalachite green. Remarkable

variations in the fingerprint region (1500 to 500 $cm^{-1}$) of the FTIR (Figure 4) spectroscopy of control malachite green and 5 h extracted metabolites indicate biodegradation of MG by *K. rosea*.

FTIR spectra of control malachite green showed the specific peaks in fingerprint region (1500 to 500 $cm^{-1}$) for the mono-substituted and para-disubstituted benzene rings which is supporting to the peak at 1585 $cm^{-1}$ for the C=C stretching of the benzene ring. Also the peak at 1170 $cm^{-1}$ for the C-N stretching vibrations and peak at 2923 $cm^{-1}$ for C-H stretching of asymmetric - $CH_3$ group gives the perception of structure of malachite green. The FTIR spectra of extracted product showed peak at 1261 $cm^{-1}$ for -C-N- stretch with supporting peak at 1098 $cm^{-1}$ and peak around 3400 $cm^{-1}$ for N-H stretch represents the formation of primary and secondary amines. The sharp peak at 800 $cm^{-1}$ for disubstituted benzene derivatives indicate aromatic nature of amines.

### 3.7. Microbial toxicity and phytotoxicity study.

Zone of inhibition was observed with control MG with all bacterial strains studied whereas its degradation products did not show growth inhibition (Table 2). These findings suggest non-toxic nature of the product formed. Previous reports showed MG degradation into leucomalachite green that is equally toxic to malachite green.[16] During phytotoxicity study, germination (%) of the both *Triticum aestivum* and *Phaseolus mungo* seeds was less with MG treatment as compare to its degradation product and distilled water. The MG than its degradation product significantly affected the length of plumule and radical indicates less toxicity of the degradation product (Table 3).

**Table 2.** Microbial Toxicity study of malachite green and its degradation product.

| Bacteria | Diameter of zone of inhibition (cm) | | | |
|---|---|---|---|---|
| | Malachite green (500 ppm) | Degradation product (500 ppm) | Malachite green (1000 ppm) | Degradation product (1000 ppm) |
| *Kocuria rosea* | 0.6 | *NI | 1.0 | NI |
| *Pseudomons aeruginosa* | 0.3 | NI | 0.4 | NI |
| *Azotobacter vinelandii* | 0.9 | NI | 1.1 | NI |

*** NI-** No Inhibition.

## 4. Conclusions

MG degradation was presumably due to reductase enzymes, as induction in activities of MG reductase and DCIP reductase was observed. MG was degraded into non-toxic compound by *K. rosea*. As previous reports showed malachite green degradation into leucomalachite green this found to be equally toxic to malachite green. This strain has also ability to decolorize other dyes including textile dyes and use of cheap source like molasses for decolorization.

## 5. Acknowledgements

Mr. G. K. Parshetti one of the authors is thankful to Shivaji University for awarding Departmental

**Table 3.** Phyto Toxicity study of malachite green and its degradation product.

| Parameters Studied. | *Triticum aestivum* | | | *Phaseolus mungo* | | |
|---|---|---|---|---|---|---|
| | Distilled water | Malachite green (100 ppm) | Extracted metabolite (100 ppm) | Distilled water | Malachite green (100 ppm) | Extracted metabolite (100 ppm) |
| Germination (%) | 90 | 80 | 90 | 100 | 70 | 90 |
| Plumule (cm) | 15.58 ± 1.23 | 1.5** ± 0.34 | 7.64** ± 0.70 | 2.12 ± 0.29 | 0.98* ± 0.15 | 2.34* ± 0.20 |
| Radical (cm) | 8.34 ± 1.17 | 0.3** ± 0.04 | 3.12** ± 0.31 | 2.82 ± 0.34 | 0.32** ± 0.05 | 1.80** ± 0.27 |

Values are mean of germinated seeds of two experiments, SEM (±) significantly different from the control (seeds germinated in distilled water) at * $P < 0.05$, ** $P < 0.001$ by one-way ANOVA with Turkey comparison test.

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## 6. References


1. S. D. Kalme, G. K. Parshetti, S. U. Jadhav, S. P. Govindwar, *Bioresour Technol. (In press).* **2006**.
2. J. P. Jadhav, S. P. Govindwar, *Yeast.* **2006**, *23*, 315–323.
3. S. Sarnaik, P. Kanekar, *Appl. Environ. Microbiol.* **1999**, *52,* 251–254.
4. S. Srivastava, R. Sinha, D. Roya, *Aqua. Toxicol.* **2000**, *66*, 319–329.
5. K. Mitrowska, A. Posyniak, *Buletin of Veerinary. Institute Pulwy.* **2004**, *48*, 173–176.
6. J. J. Jones, J. O. Falkinham, *Chemother.* **2003**, *47*, 2323–2326.
7. C. Cha, D. R. Doerge, C. E. Cerniglia, *Appl. Environ. Microbiol.* **2001**, *67*, 4358–4360.
8. V. L. Papinutti, F. Forchiassin, *FEMS Microbiol. Lett.* **2004**, *231*, 205–220.
9. M. D. Salokhe, S. P. Govindwar, *W. J. Microbiol. Biotechnol.* **1999**, *15*, 229–232
10. S. Moosvi, H. Keharia, D. Madamwar, *W. J. Microbiol. Biotechnol.* **2005**, *21*, 667–672.
11. S. B. Pointing, L. L. P. Vrijmoed, *W. J. Microbiol. Biotechnol.* **2000**, *16*, 317–318.
12. J. A. Tarbin, K. A. Barnes, J. Bygrave, W. H. H. Farrington, *Analyst.* **1998**, *123*, 2567–2571.
13. G. A. Dykes, R. G. Timm, A. V. Holy, *Appl. Environ. Microbiol.* **1994**, *60*, 3027–3029.
14. R. Russ, J. Rau, A. Stolz, *Appl. Environ. Microbiol.* **2000**, *66*,1429–1434
15. M. S. Jang, Y. M. Lee, C. H. Kim, J. H. Lee, D. W. Kang, S. J. Kim, Y. C. Lee, *Appl. Environ. Microbiol.* **2005,** *71*, 7955–7960.
16. S. Burchmore, M. Wilkinson, **1993**, United kingdom department of the Environment, Water Research Center, Marlow, Buckinghamshire, United Kingdom. *Report no. 316712 (November 1993).*


## Povzetek

Bakterija *Kocuria Rosea* MTCC 1532 je pod anoksičnimi pogoji popolnoma razbarvala malahitno zeleno (MG) v petih urah, medtem ko v stresanih kulturah razbarvanje ni poteklo. Maksimalna učinkovitost je bila dosežena pri koncentraciji 50 mg/L. Ista bakterija je razbarvala tudi nekatera azo, trifenilmetanska in industrijska barvila, ki so v polsintetskih medijih z melaso, sečnino in saharozo izginevala najhitreje. Razbarvanje, ki ga povzročajo inducirane aktivnosti MG reduktaz in DCIP reduktaz, je posledica razgradnje MG, kar dokazujejo UV-VIS spektri ter HPLC in FTIR analiza. Produkti razgradnje niso toksični.