ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Biodegradation of azo dye C.I. Reactive Orange 16 by an actinobacterium *Georgenia* sp. CC-NMPT-T3

Madhuri Sahasrabudhe[1] and Girish Pathade[2]

1. Assistant Professor, Microbiology, Maulana Azad College, Aurangabad, Maharashtra, India.

2. Head, Department of Biotechnology, Fergusson College, Pune Maharashtra, India.

Manuscript Info

Manuscript History:

Received: 13 August 2013
Final Accepted: 24 August 2013
Published Online: September 2013

Key words:
Decolourization,
Biodegradation,
Azo dyes, Toxicity,
Reactive Orange 16.

Abstract

Microbiological methods assume interest because of versatile nature of microorganisms to elaborate enzyme systems required to breakdown the chemical compounds thereby eliminating their toxic effect. In a present study, biotransformation of Reactive Orange 16, an azo dye by *Georgenia* sp. CC-NMPT-T3 was studied under static anoxic condition. It was able to decolourize 94.2% of Reactive Orange within 8 h at 50mg/L dye concentration. The selected isolate showed dye decolourization over a wide range of pH from 6-8 and temperature 28±2°C - 45°C. The biodegradation was confirmed by Uv-Visible spectrophotometry, TLC, HPLC, FTIR and GC-MS studies. Enzymatic studies revealed dye decolourization was due to a combined activity of Lignin peroxidase, laccase, NADH-DCIP reductase and azoreductase. Phytotoxicity studies demonstrated no toxicity of the degraded product. The isolate was also able to decolourize mixture of five azo dyes. Hence, this isolate could be effectively used for treatment of azo dye containing effluent.

Introduction

Dye being one of the important recalcitrant, persist for long distances in flowing water, retards photosynthetic activity, inhibit the growth of aquatic biota by blocking out the sunlight and utilizing dissolved oxygen and also decrease the recreation value of stream(Rana et al.,2013). Azo dyes are considered as electron-deficient xenobiotic compounds because they possess the azo (N=N) and sulfonic (-SO3-) electron-withdrawing groups, generating electron deficiency in the molecule and making the compound less susceptible to oxidative catabolism by bacteria. As a consequence, azo dyes tend to persist under aerobic environmental conditions (Hakim et al., 2013). Azo dyes have been used increasingly in industries because of their ease and cost effectiveness in synthesis compared to natural dyes. However, most azo dyes are toxic, carcinogenic and mutagenic (Shah et al., 2013). Many microorganisms such as bacteria, fungi and yeast have been observed to decolorize azo dyes by biosorption and biodegradation. Decolorization of dye waste water is a challenging process to the dye industry, and the great potential of microbial decolorizing can be adopted as an effective tool. Microorganisms elaborate an array of diverse enzymes which can degrade variety of organic compounds. Hence, in a present study an actinobacterium isolate *Georgenia* sp. was employed for decolourization and degradation of the selected dye Reactive Orange 16.

Material and Methods

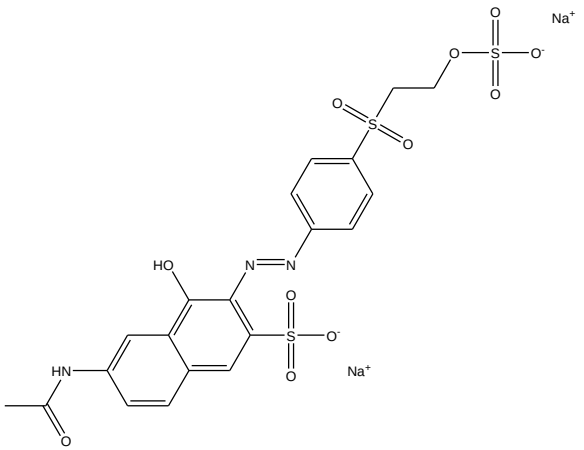
Microorganism and culture conditions:

The bacterium in the present study was isolated by enrichment culture technique by gradually adapting the isolate to the increased concentration of Reactive Orange 16(RO 16) in a basal nutrient medium (Sahasrabudhe and Pathade, 2012a) from dye industry effluent contaminated soil, sewage, dung and dye waste. Pure culture was maintained on the nutrient agar slants. Composition of nutrient broth and agar used for decolourization is (g/L) Peptic digest of animal tissue 5, NaCl 5, Beef extract 1.5, Yeast extract 1.5 and pH 7.4±0.2.

Dyestuff and chemicals:

All chemicals used were of the highest purity and of analytical grade.2'-2'Azinobis-(3 ethylbenthiazoline-

6 sulphonate) (ABTS) was purchased from Sigma Aldrich, USA. Nutrient broth dehydrated was purchased from Hi-Media, Mumbai, India. Tartaric acid, n-propanol was purchased from Qualigenes, India. The textile dye Reactive Orange 16 (RO 16) was obtained from Spectrum dyes, Surat, India.



Reactive Orange 16

Identification of the culture:
The isolate is an actinobacterium which shows rod and coccus cycle. 16s r-RNA sequencing of the isolated organism was done in GeneOmbio Technologies Pvt. Ltd., Pune, India.

Decolourization studies:
Georgenia sp.CC-NMPT-T3 was grown for 24 hrs at 37°C on nutrient agar.10% inoculum of $O.D_{600}$ 1.0(Mathew et al.,2004; Parshetti et al., 2009)was used throughout the study. Decolourization studies were carried out in nutrient broth. The dye was filter sterilized by using 0.2 µm cellulose acetate paper filter, Sartorius Biolab, Germany and added after sterilization of medium throughout the study. The dye (50mg/L) was added immediately and incubated at static condition at 37°C. The aliquot (3ml) of culture media was withdrawn at different time intervals and centrifuged at 6000g for 20 min. Decolourization with respect to time was monitored by measuring the absorbance of the culture at λ_{max} of the dye at 495 nm. In order to detect pH and temperature optima of decolourization of the dye by the isolate, nutrient broth with different pH ranging from 3-8 was inoculated with 10% inoculum at 50mg/L dye concentration and was incubated at temperatures ranging from 25-50°C. Abiotic control was always kept in each study.

Decolourization performance at different dye concentration:
Once decolourization ability of the isolate was confirmed at 50mg/L concentration, the decolourization performance of the isolate was studied at different dye concentrations from 50-500mg/L under the same experimental setup. The % decolourization was measured after every hour. All decolourization experiments were performed in three sets. Abiotic controls (without microorganism) were always included. The % decolourization was calculated as (Sartale et al., 2009; Sahasrabudhe and Pathade, 2012b).

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

Decolorization under added carbon and nitrogen sources:
Decolourization performance under added carbon and nitrogen sources was studied in semi synthetic medium described by Sartale et al., 2009. The medium was supplemented with one percent each of glucose, sucrose, lactose and starch, yeast extract, peptone, malt extract, meat extract and urea as carbon and nitrogen sources, respectively. 50 mg/L of the dye concentration used. Filter sterilized dye was added after sterilization of the medium and after inoculation of the isolate.

Preparation of the cell free extract:
Twenty four hours old culture of the isolate grown under pH and temperature optima was centrifuged at 10,000 rpm for 20 minutes. These cells were suspended in the potassium phosphate buffer (50mM, pH 7.4) and sonicated at output of 50A and giving 7 strokes each of 30sec for 12 minutes at 4°C.The homogenate was centrifuged and supernatant was used as a source of enzymes (Kalyani et al., 2008).

Enzyme assays:

Oxidative enzymes during decolourization
The activities of laccase and lignin peroxidase were assayed spectrophotometrically in the cell free extract. Laccase activity was determined in a mixture of 2 ml containing ABTS (10%) in 0.1 M acetate buffer pH 4.9 and measured as an increase in optical density at 420nm (Sartale et al., 2009;Kalyani et al.,2008 and Telke et al.,2009). Lignin peroxidase (LP) activity was determined by monitoring the formed propanaldehyde at 300nm in a reaction mixture of 2.5 ml containing 100 mM n- propanol, 250 mM tartaric acid and 10 mM H_2O_2(Parshetti et al.,2006). All enzyme assays were carried out at 37°C with reference blanks contained all components

except the assayed enzymes. All enzyme assays were conducted in triplicates and the average rates were calculated to represent the enzyme activity. One unit of enzyme activity was defined as a change in absorbance U/ min/ ml of the enzyme.

Reductive enzymes during decolourization

NADP-DCIP reductase activity was assayed by following the procedure reported by Telke et al.2009. The composition the assay mixture (5.0ml) was 25µM DCIP, 4.75ml of potassium phosphate buffer (20mM,pH 7.5) and 0.1ml of enzyme solution. The reaction was initiated by addition of 50 µM NADH. The decrease in colour intensity of DCIP was observed at 595nm.

Azoreductase activity was determined by monitoring the decrease in methyl red concentration at 440nm in a reaction mixture of 3.3 ml containing 152µM Methyl red, 50mM potassium phosphate buffer (pH 7.4) and 20µM NADH(Sartale et al.,2009) .

All enzyme assays were conducted in triplicates and the average rates were calculated to represent the enzyme activity.

Analytical procedure

The metabolites produced during the biodegradation of RO 16 at 8 hrs i.e. after decolourization of the medium were extracted twice with equal volume of dichloromethane (DCM). The DCM extracts were pooled and evaporated at 40°C in a rotary evaporator and then transferred to a test tube (Moutaoukkil et al., 2003). The extracted residue was dissolved in small volume of HPLC grade methanol and the same sample was used for analysis. During UV visible spectral analysis, changes in absorption spectrum in the decolourized medium (400-800nm) were recorded in comparison with the results from the control runs (Sartale et al., 2009). The mobile phase used for TLC was composed of methanol, ethyl acetate, n-propanol, water and acetic acid (1:2:3:1:0.2 v/v) and silica gel plates 'Merck' was used for separation. TLC plate was developed using iodine chamber (Sartale et al., 2009;Telke et al.,2009) . HPLC analysis was performed in an isocratic system Shimadzu (SCL 10 AVP) equipped with dual absorbance detector using C 18 column with HPLC grade methanol as mobile phase at the flow rate of 1.0 ml / min for 10 min (Telke et al.,2009).

The metabolites formed after decolourization of the dye by an isolate were identified by using Shimadzu GC–MS QP 2010. The ionization voltage was 70 eV. Gas chromatography was conducted in temperature programming mode with a Resteck column (0.25 mm X 9 30 mm). The initial column temperature was 40°C for 4 min, which was increased linearly at 10°C/min up to 270°C and held at 4 min. The temperature of injection port was 275°C and GC–MS interface was maintained at 300°C. The helium was carrier gas; flow rate 1 ml/min and 30 min run time (Telke *et al*., 2009).

Toxicity study

Phytotoxicity tests were carried out in order to assess the toxicity of RO 16 and metabolites formed after decolourization of RO 16. Phytotoxicity tests were carried out at a final concentration of 500 ppm on two kinds of seeds. One from grains *Sorghum vulgare* (monocot) and second from pulses *Phaseolus mungo* (dicot), commonly cultivated. Phytotoxicity was conducted at room temperature (10 seeds of each) by watering separately 5ml sample of control RO 16 and its degradation products per day. Control set was carried out using distilled water at the same time. Germination% as well as the length of plumule and radical was recorded after 7 days (Sartale et al., 2009).

Statistical analysis

Data was analyzed by one way analysis of variance (ANOVA) with Turkey- Kramer multiple comparison test. Readings were considered significant when P was ≤ 0.05.

Result and Discussion

Isolation and identification of dye decolourizing bacteria:

Isolation of bacteria was carried out by the enrichment technique using nutrient broth and RO 16 as source of carbon and nitrogen that has rapid decolourization capacity. Decolourization occurred only when a carbon and nitrogen sources were available for growth. Isolated bacterium was Gram positive facultative anaerobic motile coccus. 16sr-RNA analysis done by geneOmbio Technologies, Pune. The nucleotide alignment of this strain showed it was most phylogenetically similar to the *Georgenia* sp.CC-NMPT-T3 (Sahasrabudhe et al., 2012a).

Decolourization studies:

It is well known that decolourization of dyes by bacteria takes place in two ways, either adsorption on the microbial biomass or biodegradation of dyes by the cells. Dye decolourization can be judged clearly by observing the spectrum changes of dyes. Dye adsorption to cell reveals the approximate decrease of all peaks in proportion to each other, whereas dye removal by biodegradation shows the complete reduction of the major peaks and the production of new peaks at the same time.

The isolate showed ability to decolourize 94.2% of RO 16 within 8 hrs at a dye concentration 50 mg/L .

The absorbance peaks in the visible region disappeared indicating complete decolourization (Elisangela et al., 2009). In the UV spectra the peak at 495nm was replaced by new peak at 294.5nm.
There was no abiotic loss of RO 16 within 24 h incubation indicating that the decolourization of RO 16 was due to biological mechanism rather than adsorption. To confirm whether this decolourization was due to the bacterial action or variation in pH, change in pH was recorded in the range of 7.1±0.2.

Effect of physiochemical conditions on the decolourization performance:
The effect of various physiochemical conditions such as pH, temperature, dye concentration, effect of carbon and nitrogen sources on decolourization of RO 16 by the isolate were studied in detail. All parameters were studied at 37°C under static condition. 10% inoculum with $O.D_{600}$ 1.0 was used at a dye concentration 50 mg/L.

Effect of pH

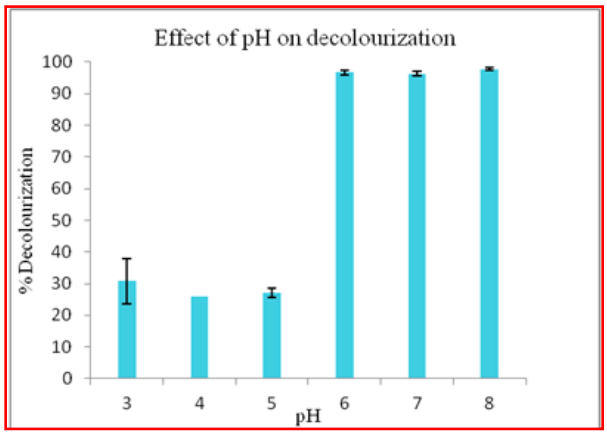


Fig 1 Effect of pH on decolourization

Bacterial cultures generally exhibit maximum decolourization at pH values near 7.0, our culture exhibited decolourization activity in the range of pH 6-8(Fig 1). At pH 3-5 decolourization observed was on an average 27.99±0.96%. The isolate showed more or less constant decolourization from pH 6 to 8, maximum 97.75% being at pH 8. Generally, bacteria show better decolourization and biodegradation activities at neutral or basic pH (Ali, 2010). Wang et al., 2009 studied decolourization of Reactive Black 5 by *Enterobacter* EC3. It showed a high decolourization rate at pH 7. Sahasrabudhe and Pathade, 2012a reported decolourization of Reactive Red 195 by *Georgenia* sp. from pH range 5-8, maximum being at pH 7.

Effect of Temperature:
Various microorganisms showed their survival at various temperatures ranging from 25°C to 50°C (Mathew et al., 2004). The isolate showed 96.175±0.198% decolourization from27-45°C but at 50°C there was 79.84% decolourization (Fig. 2). The temperature ranging between 30-40ºC was found to be suitable for the decolorization by *S.saprophyticus* strain AUCASVE3 and a further increase in the temperature gradually reduced decolorization activity of bacterial culture. This might be due to decrease in bacterial number above the optimal growth temperature (Hakim et al., 2013).

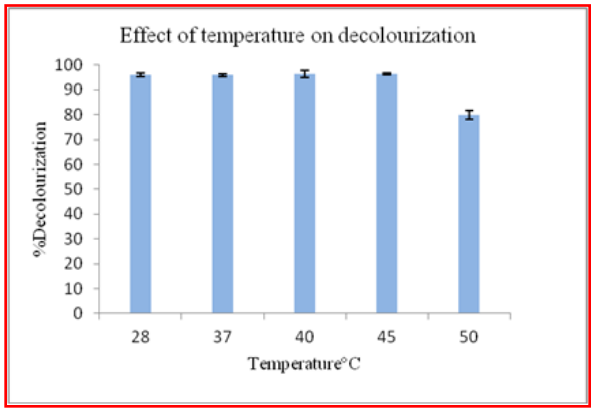


Fig 2 Effect of temperature on decolourization

Effect of initial dye concentration:
Actual concentrations of reactive dyes in dye house effluent have been reported to range from 60-250mg/L (Bhatt et al., 2005).

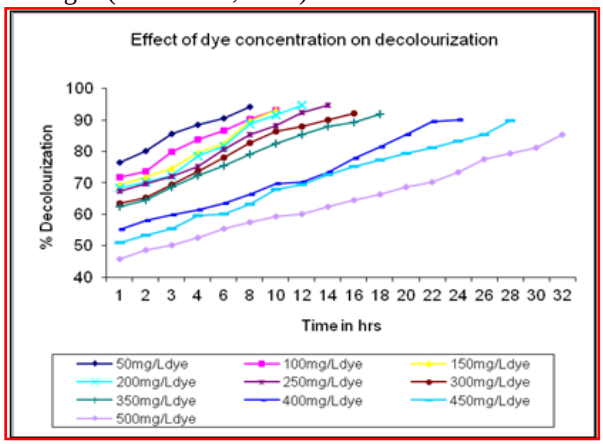


Fig 3 Effect of dye concentration on decolourization.

The isolate showed decolourizing ability up to 350 mg/L at a faster rate after which the rate began decreasing. It has been proposed that dye concentration can influence the efficiency of

microbial decolourization through a combination of factors including the toxicity imposed by dye at higher concentration (Bhatt et al.2005). The isolate could decolourize 94.20% of 50 mg/L of the dye in 8 hrs whereas it took 32 hrs to decolourize 85.24 % of 500 mg/L (Fig 3). Thus, the isolate which could decolourize dye up to the reported dye concentration in wastewater, can be successfully employed for treatment of dye bearing industrial wastewater.

Effect of carbon and nitrogen sources:

While trying to enhance decolourization performance of RO 16, extra carbon and nitrogen sources was supplied in semi synthetic medium. There was no decolourization in the presence of synthetic media. There was no enhanced decolourization in presence of urea, glucose and starch. Percentage decolourization was maximum with yeast extract and sucrose 97.15% and 96.46% respectively within 24 h (Fig 4). Similar results were also reported by bacterial consortium RVM for decolourization of reactive violet 5 (Moosvi et al., 2007). The culture showed moderate decolourization in the presence of sucrose (51.21%) lactose (42.04%) and starch (52.00%) and maximum decolourization was reported in presence of glucose (65.88%). In addition, supplying urea as a nitrogen source exhibited less decolourizing ability (41.57%). In contrast, addition of carbon sources seemed to be less effective to promote the decolourization probably due to the preference of the cells in assimilating the added carbon sources over using the dye compound as the carbon source. Shah et al., 2013 reported percentage decolorization (95%) was maximum with purified substrate peptone while less decolorization with other supplements of carbon and nitrogen source in degradation of Ramazol Black B by *Bacillus* ETL-2012.

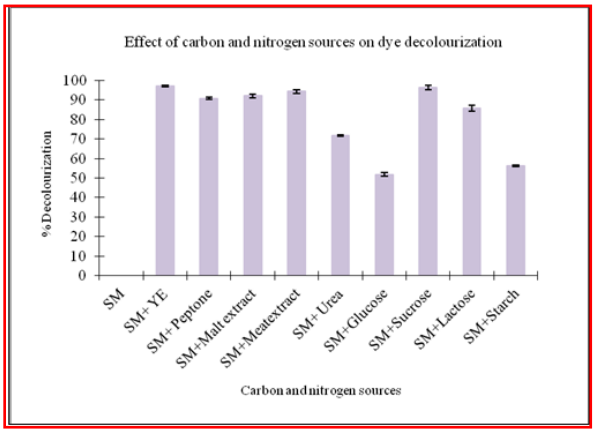


Fig 4 Effect of carbon and nitrogen sources on decolourization

Analysis of metabolites resulting from decolourization and biodegradation of RO 16 by *Georgenia* sp.CC-NMPT-T3:

In order to understand the possible mechanism of the dye decolourization, we also analyzed the products of degradation of RO 16 by UV visible spectral analysis, TLC, HPLC, FTIR and GC-MS.

UV visible scan (400-800nm) of the culture supernatant withdrawn at different time intervals indicated the decolourization and decrease in dye concentration from batch culture. Peak obtained at 495 nm decreased at complete decolourization. The absorbance peak in the visible region disappeared indicating complete decolourization. In the UV spectra, the peak at 495 nm was replaced by new peak at 294.5nm.

TLC analysis showed the appearance of one spot in the sample containing the extracted metabolites of completely decolourized medium with Rf value 0.81 wheres Rf value of RO 16 was noted as 0.92 confirming the biodegradation of RO 16 by *Georgenia* sp.CC-NMPT-T3.

HPLC

HPLC elution profile of RO 16 showed prominent peak at retention time 1.65, 1.94, 2.93and 3.39 min while degradation products by *Georgenia sp.* show 2.668, 2.741, 2.912, 3.008, 3.467 and 3.851 min. The analysis showed the presence of new peak with disappearance of the peaks of RO 16 confirming the degradation of the dye by *Georgenia* sp.CC-NMPT-T3. **(**Fig 5 A and Fig 5 B).

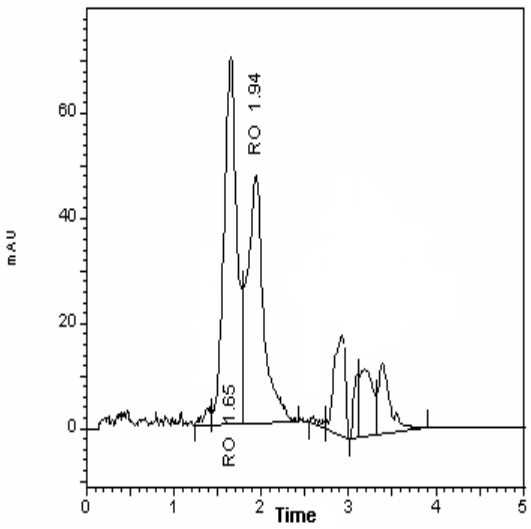


Fig 5 A HPLC of RO 16

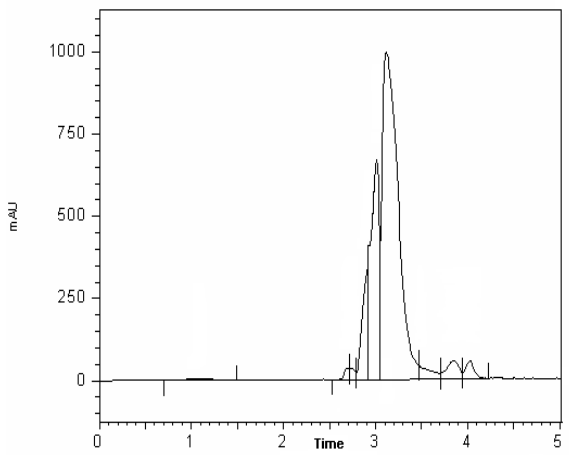


Fig 5B HPLC of degraded products

FTIR

Jadhav *et al.* (2010) reported that RO16 showed specific peaks in the fingerprint region for distributed and multisubstituted benzene and naphthalene ring which is supported by the peaks of 628 cm-1 for C-C bending vibrations, 664 cm-1 for S=O stretching for sulphonic acid. 830 cm-1 and 895 cm-1 for C-H bending vibrations. The group frequency region shows specific peaks for functional groups. The peaks at 1496 cm-1 for C=C stretching vibrations of benzene ring, 1671 cm-1 for N=N stretching vibrations 1853, 2924 for C-H stretching vibrations and –CH3 and –CH2 group, 3435 cm-1 for –NH stretching vibrations. FTIR of RO16 in the present study showed phenolic O-H and N-H stretch at 3385.42 cm-1, N=N azo group at 1664.27 cm-1, 1500 cm-1 showed N-H bending and C=C ring, 1235cm-1 indicates C-N stretch, 1049 cm-1 showing S=O stretch whereas 831 cm-1, 612.288 cm-1 indicating C-H bending and C-C bending vibrations. FTIR spectrum of the extracted metabolites showed absence of azo group and appearance of new groups confirming dye degradation.

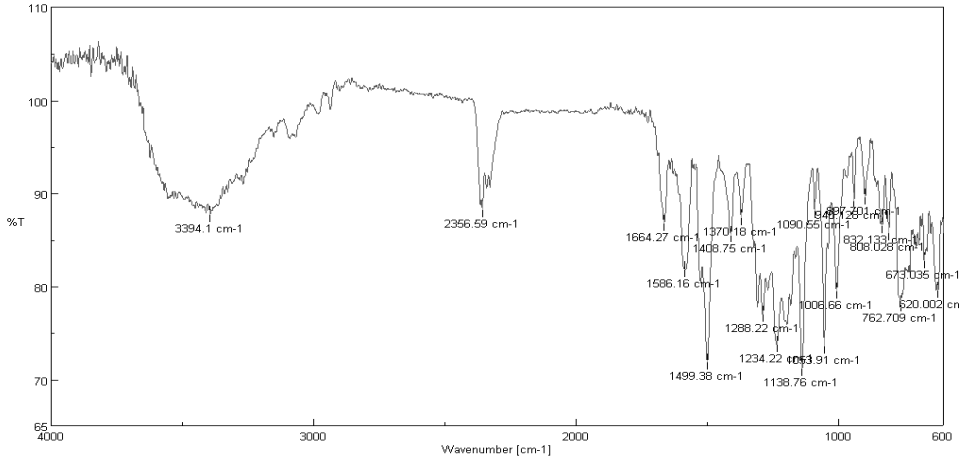


Fig 6 A FTIR of RO16

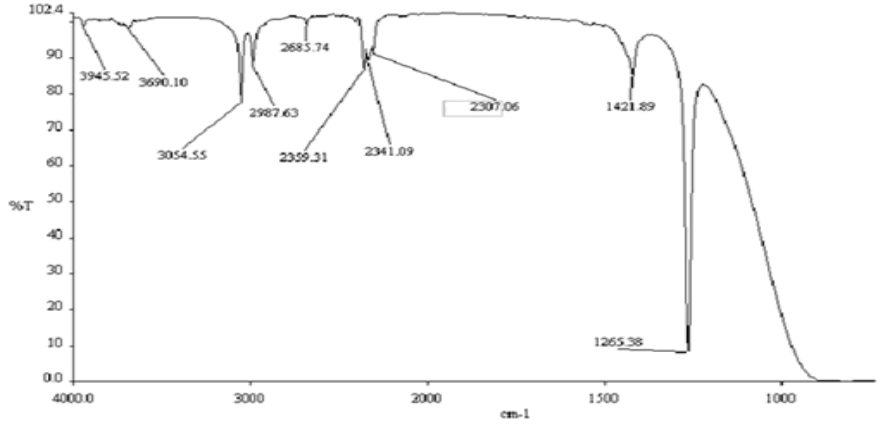


Fig 6 B FTIR of degradation products

Enzyme assays:

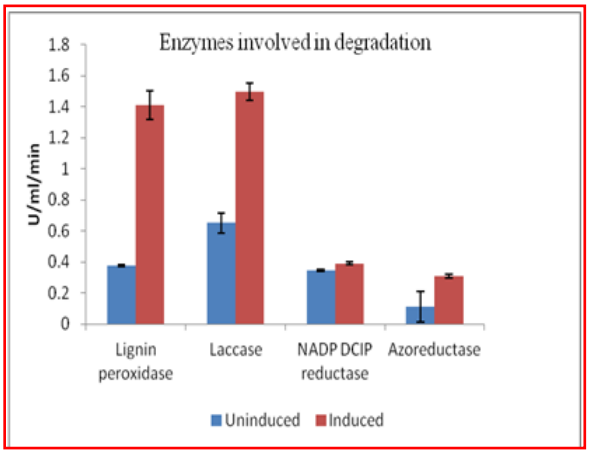


Fig 7 Enzymes involved in degradation

In order to get the details of the enzymes involved in decolourization mechanism lignin peroxidase, laccase and NADP-DCIP reductase were monitored after complete decolourization. No activities of enzymes were observed in the cell free supernatant. Enzymatic profile of uninduced cells and induced cells of *Georgenia* sp.CC-NMPT-T3 obtained after complete decolourization was studied (Fig 7). Enzyme profile showed enhanced activity of Laccase, Lignin peroxidase and azoreductase. It can be presumed that the major mechanism of decolourization in the cells is mostly because of the biotransformation enzymes i.e. laccase, lignin peroxidase and azoreductase. The relative contribution of enzymes to decolourization of dye may be different for each microorganism. Telke et al., 2009 also reported significant induction in NADH-DCIP reductase after 24 h of decolorization of Reactive orange 16 by isolated *Bacillus* strain.
Similarly bacteria are also able to produce peroxidase, which can degrade azo dyes. The induction of these enzymes suggests the prominent role of these enzymes in the decolourization process. This supports the earlier observations.

Phytotoxicity study:

Despite the fact, untreated dyeing effluent may cause the serious environmental and health hazards. They are being disposed off in the water bodies and this water can be used for irrigation purpose. Thus it was found necessary to access the phytotoxicity of the dye before and after degradation. The relative sensitivities towards the dye RO 16 and its degradation products in relation to *Sorghum vulgare* and *Phaseolus mungo* seeds were presented in the Fig 8 A and Fig 8B, respectively.

There is no significant difference in the root and shoot length in case of *Sorghum vulgare* irrigated with the dye but in case of metabolite irrigated, the root and shoot length was significantly increased ($p \leq 0.05$)as compared to control wheat.
There is no significant difference in the root and shoot length in case of *Phaseolus mungo* irrigated with the dye and the root length of mung seeds irrigated with metabolite but in case of metabolite irrigated ,shoot length was significantly increased ($p \leq 0.05$)as compared to control mung seed.
Phytotoxicity study showed good germination rate as well as significant growth in the plumule and radical for both the selected plants ($P \leq 0.05$) in the metabolites extracted after decolourization as compared to dye sample. This indicates the detoxification of RO 16 by the isolated bacterial culture.

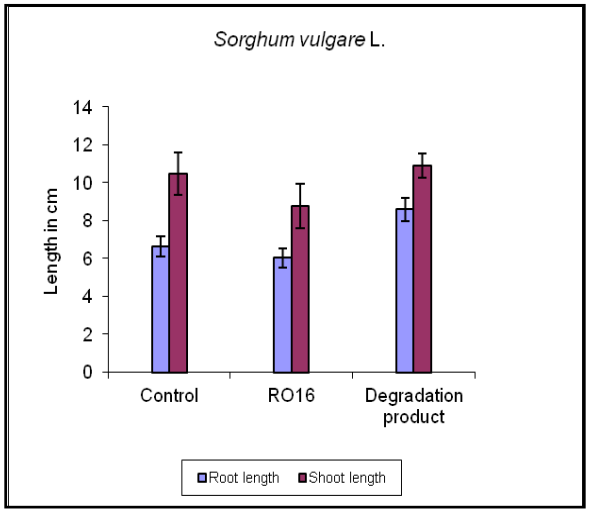


Fig 8A Phytotoxicity using *S.vulgare*

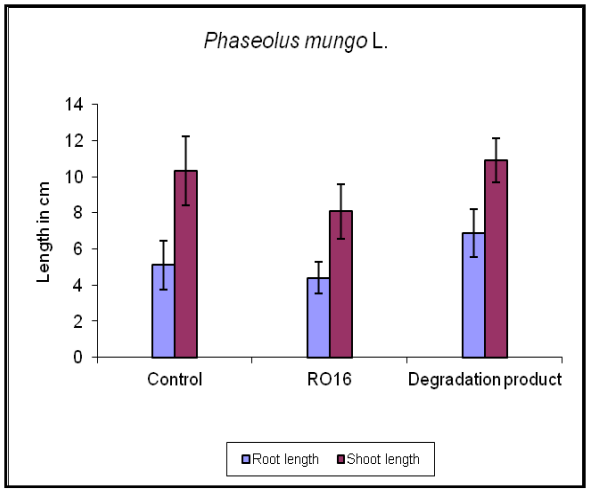


Fig 8 B Phytotoxicity using *P.mungo*

GC-MS analysis of degradation products:

Reactive Orange 16 degradation products suggested the breakdown of azo bond. Azo dyes can be cleaved symmetrically or asymmetrically, with an active site available for an enzyme to excite the molecule. The induced status of lignin peroxidase after 12 h of decolorization and formation of 6-acetamido naphthol 2-sulfonic acid and hydroperoxy ethyl sulfone phenyl dizonium intermediates suggested asymmetric cleavage of C.I. Reactive Orange 16 by lignin peroxidase enzyme. Significant induction of NADH–DCIP reductase after 24 h of decolorization and formation of dihydroperoxy benzene and 6-nitroso naphthol intermediates suggested reduction of intermediate metabolites (Telke et al., 2009).

In the present study,(Fig 9) a peak at m/z value of 279 was identified as 6 acetamido naphthol 2 sulfonic acid whereas a peak at m/z value of 170 indicates 6-niitroso naphthol confirming degradation of Reactive Orange 16 in small molecular weight compounds. Similar results were reported by Telke et al., 2009.

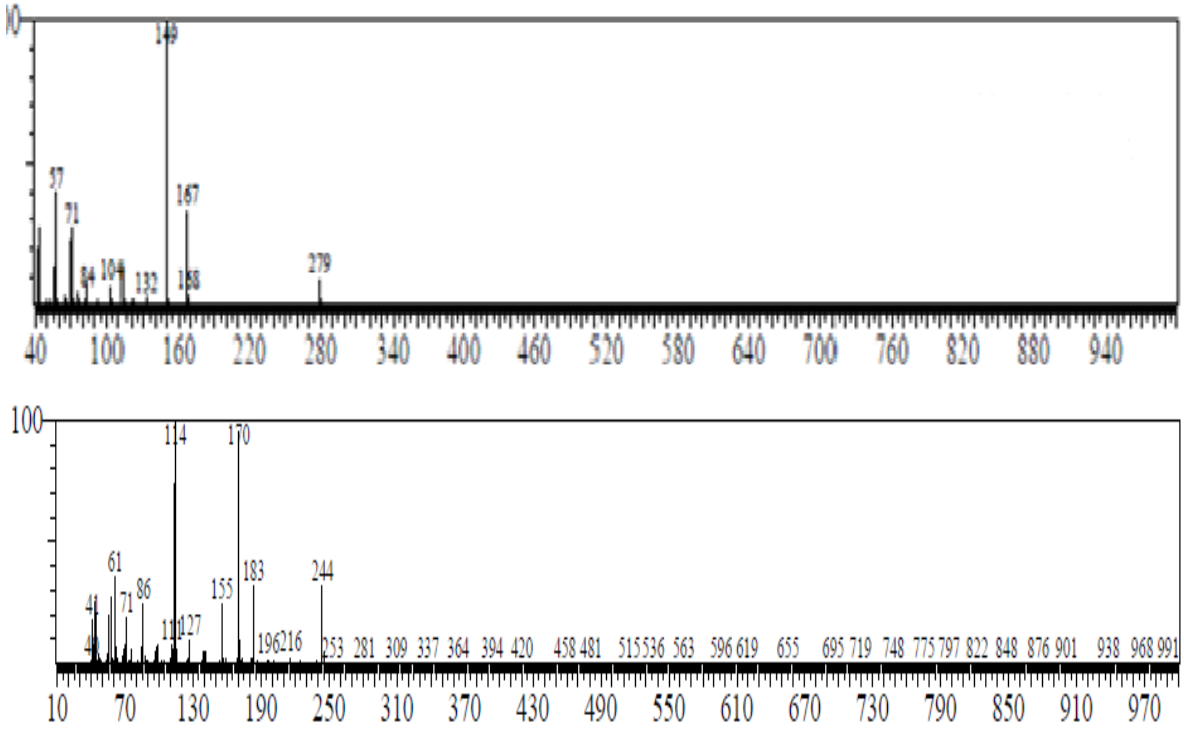


Fig 9 GC-MS analysis of the degradation products

Hence this indigenous bacterial strain could be utilized for treatment of dye containing wastewater.

References

1. Ali, N., Hameed, A., Ahmed, S. and Khan, A. G. (2008): Decolourization of structurally different textile dyes by *Aspergillus niger* SA1. World J. Microbiol. Biotechnol.,24: 1067-1072.

2. Bhatt, N., Patel, K.C., Keharia, H. and Madamwar D. (2005): Decolourization of diazo dye Reactive blue 172 by *Pseudomonas aeruginosa* NBAR12. J.Basic Microbiology., 46 : 407-418.

3. Elisangela, F., Andrea, Z., Dias G.F, Cristiano, R., Durrant, R. and C:.P. Artur (2009) Biodegradation of textile azo dye by a facultative *Staphylococcus arlettae* VN-11 using a sequential microaerophilic/aerobic process. Int. Biodeter.Biodegra., 63:280-288.

4. Hakim, A., Hoque, S. and Ullah, S.M. (2013): Characterization and identification of methyl orange decolourizing bacteria from textile effluent. Adv. Agri. Sci. Engi. Res.3:918-923.

5. Jadhav, J.P., Kalyani, D.C., Telke, A.A., Phugare, S.S. and Govindwar, S.P.(2010): Evaluation of the efficacy of a bacterial consortium for the removal of colour, reduction of heavy metal, and toxicity from textile dye effluent. Bioresour Technol 101:165-173.

6. Kalyani, D.C, Patil, P.S., Jadhav, J.P. and Govindwar, S.P.(2008):Biodegradation of reactive textile dye Red BLI by an isolated bacterium *Pseudomonas* sp. SUK1. Bioresour. Technol.,99: 4635-4641.

7. Mathew, S. and Madamwar, D. (2004): Decolourization of Ranocid Fast Blue dye by bacterial consortium SV5. Appl. Biochem. Biotechnol.,118: 371-381.

8. Moosvi, S., Keharia, H. and Madamwar, D.(2007): Decolourization of textile dye Reactive Violet 5 by a newly isolated bacteria consortium RVM 11.1. World J. Microbiol. Biotechnol.,21:667-672.

9. Moutaouakkil A., Y. Zeroual, F.Z. Dzari, M.Talbi, K.Lee .M.Blaghan.(2003): Bacterial degradation of azo dye Methyl red by *Entrobacter agglomerans*. Annals of Microbiology., 53: 161-169.

10. Parshetti G.K., Kalme S.D., Sartale G.D. and Govindwar S.P.,(2006): Biodegradation of malachite green by *Kocuria rosea* MTCC 1532. Acta Chim. Slov., 53:492-498.

11. Parshetti G.K., Sartale, G.D., Telke, A. and Govindwar, S.P.(2009): Biodegradation of hazardous triphenylmethae dye methyl violet by *Rhizobium radiobacter* MTCC 8161. J. Basic Microbiol., 49:36-42.

12. Rana, J. and Kumar, S. (2013): A Comparitive Study of Adsorption Behaviour of a Dye Using Agro Wastes as Adsorbents. IOSR-JESTFT, 4: 91-95.

13. Sahasrabudhe, M. M. and Pathade, G. R. (2012a): Decolourization and degradation of C.I.Reactive Red *Georgenia* Sp. CC-NMPT-T3. Ind.J.Exptal.Bio., 50:290-299.

14. Sahasrabudhe, M. M. and Pathade, G. R. (2012b): Biodegradation of C.I.Reactive Red 195 by *Enterococcus faecalis* strain YZ66.World J. Microbiol. Biotechnol. 28:815-826.

15. Saratale R.G, Saratale, G.D., Chang, J.S. and Govindwar, S.P.(2009):Ecofriendly degradation of sulphonated diazo dye C.I. Reactive Green 19A using *Micrococcus glutamicus* NCIM-2168. Bioresour. Technol., 100:3897-3907.

16. Shah, M., Patel, K.A., Nair, S.S. and Darji, A.M.(2013): Microbial degradation of textile dye (Ramazol Black B) by *Bacillus* spp. ETL-2012. J. Bioremed. Biodeg.4:186.

17. Telke A.A., Kalyani, D., Dawkar, V. and Govindwar, S. (2009): Influence of organic and inorganic compounds on oxidoreductive decolourization of sulfonated azo dye C.I.Reactive Orange 16. J. Hazard. Mater., 172:298-309.

18. Wang,H., Zheng,X., Su,J., Tian, Y., Xiong, X. and Zheng, T.(2009):Biological decolourization of the reactive dyes Reactive Black 5 by a novel isolated bacterial strain *Enterobacter* Sp.EC3. J.Hazard.Mater., 171:654-659.